THE IMPACT OF NUTRITIONAL AND INFLAMMATORY BIOMARKER LEVELS ON CD4 RECOVERY TO 96 WEEKS IN A TREATMENT-NAÏVE HIV-POSITIVE COHORT

by
Erin R. Ewald

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ABSTRACT

Background

As antiretroviral therapy (ART) becomes more widespread globally, it is important to investigate factors which have an effect on HIV progression in order to determine the best intervention for individual patients. Nutritional and inflammatory factors have recently been at the forefront of this investigation; however, their effect on CD4 recovery is not well-studied.

Objectives

This study aims to determine nutritional and inflammatory markers that have an effect on CD4 recovery in a treatment-naïve HIV-positive cohort consisting of individuals in nine low-, medium-, and high-income countries.

Methods

469 adults initiating ART in nine countries with CD4 count of <350 cells/mm^3 were used for this analysis. Associations between baseline levels of nutritional and inflammatory biomarkers and longitudinal CD4 recovery were measured using a random-effects generalized least squares (GLS) linear model at 24 and 96 weeks. Logistic regression using generalized estimating equations (GEE) was used to examine the longitudinal relationship between CD4 recovery and specific nutritional deficiencies at 96 weeks.

Results

Multivariate GLS analysis at week 24 revealed statistically significant associations between longitudinal CD4 recovery and low baseline levels of β-carotene and lycopene,

as well as 25(OH)-Vitamin D deficiency (decrease of 14.07 CD4 cells, p=0.027). At 96 weeks, we observed statistically significant associations between CD4 recovery and lower levels of α-carotene and lycopene, increased levels of sCD14, and selenium deficiency (decrease of 24.01 CD4 cells, p=0.012). 25(OH)-Vitamin D deficiency was also trending significant (decrease of 17.89 CD4 cells, p=0.059).

Using an adjusted GEE logistic regression model at 96 weeks, we found that Vitamin B12 deficiency was associated with a higher odds of CD4 non-recovery (OR=2.00, p=0.031); selenium deficiency was also significantly associated with worse CD4 recovery, with a p-value of 0.024 (OR=1.54, p=0.024).

Conclusions

Our results show that baseline levels of nutritional biomarkers are associated with CD4 recovery over time after ART initiation, especially selenium and 25(OH)-vitamin D. Although achieving optimal CD4 recovery after ART initiation remains a challenge in many areas of the world, understanding the relationship between nutritional deficiencies and CD4 recovery can help us to identify and appropriately monitor high-risk populations.

Thesis Readers:

Jonathan E. Golub, MPH PhD

Amita Gupta, MD

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INTRODUCTION

Human immunodeficiency virus (HIV) is one of the most devastating diseases for low- and middle-income countries, which accounted for 97% of the estimated 35.5 million HIV-positive individuals in 2012 [1]. An important indicator of HIV progression is a decrease in $CD4^+$ T lymphocyte count (referred here as CD4 count); because HIV preferentially infects CD4 cells, CD4 count provides an immunologically relevant measure for disease progression [2]. CD4 recovery after initiation of antiretroviral therapy (ART) is essential for restoring immune function in HIV-positive individuals; however, CD4 recovery is variable in different individuals and many factors have been found to affect recovery. Differences by sex [3][4], race [5], geographic region [5], intravenous drug use [6], and alcohol use [7] can each affect the level of CD4 recovery, and their effects can vary by individual. Nutritional and inflammatory biomarkers are also currently being investigated for their relationship to HIV progression due to their potential antioxidant effects and effects on immune function. However, the roles of nutritional, inflammatory, and immune activation biomarkers in CD4 recovery after ART initiation have not been well studied, especially in low- and middle-income populations.

Because much of the HIV epidemic is focused in areas with populations experiencing chronic undernourishment, the identification of specific nutritional and inflammation-related risk factors related to nutrition beyond BMI is a clear priority, as the combination of undernourishment and HIV infection is thought to have an additive negative effect [8]. The combination of this knowledge and the ever-improving tools to measure nutritional deficiencies has led to an increase in studies using serum biomarkers to identify risk

factors for worse HIV progression. Neopterin and β-2microglobulin, both serum biomarkers of immune activation, have been identified as strong predictors of HIV disease progression [9], and VCAM-1, a marker for endothelial activation, has been seen to be predictive of the risk of HIV-1 progression as well [10].

Acute HIV infection is facilitated by the binding of the virus' gp120 molecule to the CD4 receptor on CD4 cells, as well as the binding of HIV's gp20 molecule to the co-receptors CCR5 and CXCR4 [11]. Response of CD4 cells is known to be an important factor in the immune system's ability to fight a viral infection, as these cells are responsible for sending signals to other areas of the body to recruit additional immune cells to fight the infection [12]. An initial decrease in CD4 cells shortly after the initial infection is followed by a rapid increase in the number of circulating CD4 cells soon after this depletion in response to the viral infection. This early CD4 activity is critical for the early containment of the virus and has been associated with an initial decrease of plasma viral load[12][13], However, this burst of immune activation can also have detrimental effects – it is hypothesized that persistent immune system hyperactivation can lead to the destruction of the naïve CD4 cell pool and CD4 cell depletion [14]. Over time, HIV-positive individuals experience a decrease and eventual depletion of CD4 cells. Time to CD4 depletion varies by patient but is affected by factors such as HIV viral load and time of treatment initiation, if any [15].

Because CD4 cells are preferentially targeted for infection by HIV, a depleted CD4 count is a typical characteristic of HIV-related immunosuppression [16][17]. CD4 depletion is

achieved via multiple pathways, from both direct (mediated by HIV) and indirect (mediated by the immune system) action of HIV; the two principal causes are destruction of mature CD4 cells and impaired production of naïve CD4 cells [16]. Destruction of mature CD4 cells is mediated by HIV-induced apoptosis, disruption of cell membranes, accumulation of unintegrated viral DNA, cytolysis and apoptosis by cytolytic T-cells or natural killer (NK) cells that recognize an HIV-infected cell, and autoimmune reactions derived from the humoral immune system, among other mechanisms [16]. Production of naïve CD4 cells can be impacted by HIV-mediated death of CD4 progenitor cells, disruption of immune signaling and cytokine production for cell production, and nonspecific HIV-related health problems and co-infections that cause decreased functionality of the immune system [16]. Because CD4 count is an immunological indicator of HIV disease progression, it is often used to determine treatment options; currently, the World Health Organization (WHO) recommends that all HIV-positive patients with CD4 count $\leq$500 cells/mm^3 initiate ART [15][18].

CD4 recovery after initiation of ART is a critical feature of restoring immune function in HIV-positive individuals. Monitoring patients' CD4 changes once ART is started is an important indicator of how the patient is responding to the treatment; an increase of approximately 100 cells/mm^3 is expected the first year on treatment, followed by subsequent increases of approximately 50 cells/mm^3 per year after the first year [19][20]. Immunologic failure occurs when a patient fails to reach these CD4 benchmarks while simultaneously achieving viral suppression [20]. However, there is no formal consensus among clinicians as to what exactly defines an immunologic failure; immunologic

response is often complicated by AIDS-defining co-morbidities and the baseline CD4 count of a patient, making one generalizable definition difficult [20].

The overall objective of this study was to determine the effect of specific micronutrient deficiencies and elevated inflammatory biomarkers at treatment initiation on CD4 recovery from both a short-term and a long-term standpoint. This information could be used in a clinical setting to determine the most effective combination of treatment and nutritional supplementation to keep CD4 counts as high as possible over time after treatment initiation.

METHODS

Study Description & Population

Our study population was a stratified sample by country of the Prospective Evaluation of Antiretrovirals in Resource Limited Settings (PEARLS) trial, a randomized clinical trial examining once-daily versus twice-daily dosing of three ART regimens (AIDS Clinical Trials Group A5175, clinicaltrials.gov NCT00084136). In the parent trial, a total of 1,571 participants were recruited between May 2005 and July 1007 from ACTG sites in Rio de Janeiro and Porto Alegre, Brazil (n=231, 14.7%); Port-au-Prince, Haiti (n=100, 6.4%); Chennai and Pune, India (n=255, 16.2%); Blantyre and Lilongwe, Malawi (n=221, 14.1%); Lima, Peru (n=134, 8.5%); Durban and Johannesburg, South Africa (n=210, 13.4%); Chiang Mai, Thailand (n=100, 6.4%); Harare, Zimbabwe (n=110, 7.0%); and all ACTG sites located in the United States (n=210, 13.4%). ACTG Sites in the United

States were located in major metropolitan areas, and individuals enrolled at these locations accounted for approximately 11.2% of all participants at baseline.

In the parent trial, eligibility for inclusion was determined as ≥18 years, confirmed HIV-1 infection, CD4 count of <300 cells/mm^3, and less than one week of documented cumulative antiretroviral therapy at baseline. Potential participants with abnormally high or low values of absolute neutrophils, hemoglobin, creatinine clearance, or aspartate transaminase or alanine transaminase were excluded from the study. No pregnant women were included in the study; women of reproductive potential agreed to use at least one form of contraception. Enrollment of participants occurred via a centralized web-based system managed by the ACTG Data Management Center. Participants were randomized equally to the three treatment regimens: 1) efavirenz with twice-daily lamivudine-zidovudine; 2) atazanavir with didanosine EC and emtricitabine (all given once daily); and 3) efavirenz with emtricitabine-tenofovir-DF once daily.

For this analysis, we used a sample of 469 individuals chosen from the full cohort of 1,571 individuals. These 469 individuals consist of a previously chosen random subcohort from the parent trial (n=269) plus an additional 200 individuals for whom baseline biomarker data was available; these individuals were included to increase the statistical power of the analysis. In order to establish that these individuals were representative of the individuals included in the random subcohort, we compared their demographics and baseline biological characteristics and found no significant differences between groups.

This study was approved by the Institutional Review Board at the Johns Hopkins School of Medicine as well as at local Institutional Review Boards in all other countries.

Data Collection and Laboratory Analysis

Participants at baseline underwent a thorough physical exam, medical history review, serum chemistries, liver function tests, CD4 count, and plasma HIV-1 RNA viral load. Serum collected at the baseline visit was stored at -80°C, and plasma HIV-1 RNA viral load was measured using Roche Amplicor Monitor Assay (v1.5, Branchburg, NJ). Body mass index (BMI) was calculated at baseline using participants' height in meters and weight in kilograms.

CD4 count was measured at study entry and at weeks 4, 8, and every eight weeks thereafter through week 96. Levels of nutritional and inflammatory markers were measured at baseline; all biomarker levels were measured at ACTG laboratories that were externally assured as part of the NIH Division of AIDS and ACTG Network lab quality assurance [21].

Micronutrient concentrations were measured at a CLIA-certified laboratory at the Johns Hopkins Hospital (Baltimore, MD). Vitamin B_{12} was measured using Abbott AxSYM (Abbott Canada, Quebec, Canada), and vitamin B_6 levels were measured by high performance liquid chromatography-fluorescence detection (HPLC-FD). All antioxidants and fat-soluble vitamins (vitamin A, α-tocopherol, γ-tocopherol, α-carotene, β-carotene,

β-cryptoxanthin, lutein, zeaxanthin, and lycopene) were measured using high performance liquid chromatography-ultraviolet detection (HPLC-UV). Serum 25(OH)-vitamin D levels were using a chemiluminescence immunoassay (DiaSorin, Stillwater, MN), which reports a total serum 25(OH)-vitamin D concentration. Selenium concentration was measured using a Perkin-Elmer AAnalyst 600 graphite furnace atomic absorption spectrometer. Ferritin was measured using the Alpco Ferritin ELISA kit (ALPCO Diagnostics, Salem, NH), and soluble transferrin receptor and C-reactive protein (CRP) were measured using the Quantikine ELISA kit (R&D Systems, Minneapolis, MN).

Inflammatory markers other than CRP were measured at the University of California, Davis School of Medicine (Davis, CA). LPS was measured using the Limulus Amebocyte Lysate assay (Lonza, Walkersville, MD), and soluble CD14 and endocab IgM were measured using ELISA assays (R&D Systems, Minneapolis, MN; Hycult Biotechnology, Plymouth Meeting, PA). Interferon-γ, TNF-α, and IL-6 were measured using the MILLIPLEX MAP Human High Sensitivity Cytokine/Chemokine Multiplex Assay (Millipore, Billerica, MA), and IP-10 was measured with the Human IP-10 V-PLEX Kit (Meso Scale Discovery, Rockville, MD).

Variable definitions and coding for analysis

Vitamin B12 and soluble transferrin receptor values were converted into SI units before analysis [22]. Deficiency measures for nutritional biomarkers were defined in accordance with prior publications and clinically relevant standards. Vitamin B_{12} deficiency was

defined as <148 pmol/L; vitamin B_6 deficiency was defined as <19 nmol/L; vitamin A deficiency was defined as <0.7 umol/L; ferritin deficiency was defined as <12 ug/L; soluble transferrin receptor deficiency was defined as >8.3 mg/L; and selenium deficiency was defined by serum selenium levels of <85 ug/L [23][24]. Deficiency measures for soluble transferrin receptor-ferritin index (>1.8) indicate iron deficiency [25]. 25(OH)-vitamin D deficiency was defined as <32 ng/ml, with further categories of insufficiency (20-32 ng/ml), moderate deficiency (10-<20 ng/ml), and severe deficiency (<10 ng/ml), as seen in previous publications [26]. However, because there were no significant differences between the levels of deficiency we used 25(OH)-vitamin D as a dichotomous variable with a threshold of 32 ng/ml. LPS levels were considered elevated if LPS could be detected in the serum sample; otherwise if no LPS was detectable in the sample, LPS was considered within a normal range. Although there is an accepted deficiency measure for α-tocopherol (<9.3 μmol/L), only one individual was deficient (in Malawi); thus, we analyzed α-tocopherol in quartiles as described below.

For all biomarkers without defined levels of deficiency, including all immune activation markers except LPS, quartiles were used to analyze the effect of baseline levels on CD4 reconstitution. Nutritional biomarkers were analyzed using the highest quartile (Q4) of baseline biomarker as a reference, and results are presented in order of decreasing quartile, with Q1 containing the lowest values for each biomarker (the most deficient samples). Inflammatory markers and plasma cytokines used the lowest quartile (Q1) as a reference since elevated levels of these biomarkers have been seen to be associated with disease progression [27][28]. Cutoffs for quartiles for each variable can be seen in

Appendix B, and Lowess plots measuring the CD4 recovery by demographic variables and biomarkers can be found in Appendices C and D, respectively.

A variable for BMI was calculated using each participant's weight and height at baseline using the formula: BMI = weight (kg) / height^2 (m). In the analysis, we used BMI as a categorical variable in accordance with the WHO International Classification system. A BMI of ≤18.5 is categorized as underweight; a BMI of >18.5 and ≤25 is considered within the normal BMI range; and a BMI >25 is categorized as overweight [29]. Ninety-six individuals had BMI>25; because most of those individuals were not considered obese by WHO standards (BMI>30), we did not further categorize the data into overweight and obese categories.

Age was used as a dichotomous variable, with a cutoff point of 40 years based on the literature discussing the impact of age on CD4 count and HIV progression [30]. Race was coded in four non-overlapping categories: white, black, Hispanic, and Asian. One individual from the United States who identified as more than one race was dropped from the analysis. Demographic risk factors for established nutritional deficiencies were assessed using Fisher's exact test, and baseline biological risk factors were assessed using a t-test for means stratified by nutritional deficiency.

Longitudinal Data Analyses

First, the effect of baseline levels of biomarkers on CD4 recovery was assessed using a random-effects generalized least squares (GLS) linear model. In order to assess early and

late effects of biomarkers, the outcome variable was the absolute change in CD4 count at 24 (“early’) and 96 weeks (“late”), measured continuously. The GLS random-effect model was used to fit regression models to panel data while allowing for subject-specific effects, and modeled the change in the outcome variable (i.e, continuous CD4 count) in relation to other covariates. As previously described, in our analysis we used biological thresholds for biomarkers with accepted deficiency levels and quartiles for biomarkers without accepted deficiency measures. Covariates were included in the adjusted models if they were found to be significant on univariate analysis ($p<0.05$) or known to be associated from previous studies (shown in Appendix A). Missing data was managed using model-wise deletion.

An additional logistic regression analysis was conducted to observe the association between established nutritional deficiencies and CD4 recovery. In the regression models, CD4 recovery was defined as an increase of ≥50 cells at 24 weeks, ≥100 cells at 48 weeks, and ≥200 cells at 96 weeks, based on previous literature [31][32]. Recovery was assessed at each of these timepoints using a binary variable for recovery, and generalized estimating equations (GEE) with an exchangeable correlation matrix was used to examine the longitudinal relationship between CD4 recovery and selected nutritional deficiencies. This analysis was included to incorporate common thresholds for CD4 recovery used by clinicians in order to investigate more clinically relevant changes in CD4 count. All analyses were conducted using STATA, version 12.1 (StataCorp LP, College Station, TX) with a significance level of $\alpha=0.05$ or less.

RESULTS

Characteristics of the study population

The study population's characteristics (n=469) are described in Table 1. The median age at study entry was 35 (IQR 29-40). The overall population was divided fairly evenly between the sexes, with 254 males (54.2%) and 215 females (45.8%). When considering country of origin, 45 individuals were from Brazil (9.6% of the population); 38 individuals were from Haiti (8.1%); 107 individuals were from India (22.8%); 62 individuals were from Malawi (13.2%); 36 individuals were from Peru (7.7%); 58 individuals were from South Africa (12.4%); 37 individuals were from Thailand (7.9%); 48 individuals were from the United States (10.2%); and 38 individuals were from Zimbabwe (8.1%). 5.5% were white non-Hispanic, 47.3% were black non-Hispanic, 16.0% were Hispanic, and 31.1% were Asian. The majority (67.2%) fell within the normal BMI range, with 58 individuals (12.4%) categorized as underweight and 90 individuals (20.5%) categorized as overweight or obese. Almost one-fourth (23.9%) had TB disease at baseline. Mean (SD) baseline hemoglobin levels were 12.1 g/dl (1.99 g/dl). The average CD4 count at baseline was 153.2 (SD 84.6) cells/mm^3, and the average viral load at baseline was 218192.6 (SD 229007.9) copies/mL. The individuals included in our study population that were outside of the random subcohort were not found to be significantly different than the comparable individuals in the random subcohort based on demographic and biological factors.

Prevalence of nutrient deficiencies in HIV-infected adults

Baseline serum samples from the full cohort of ART-naïve HIV-infected individuals (n=469) were tested for concentrations of vitamin A, vitamin B12, vitamin B_6, 25(OH)-vitamin D, vitamin E (α-tocopherol and γ-tocopherol), soluble transferrin receptor, selenium, α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene. Approximately 12% of the samples for each biomarker were not able to be tested due to low plasma volume or regulatory complications with exporting samples from India; these missing samples were excluded from the analysis. We measured prevalence of nutrient deficiencies in the cohort among those nutrients with accepted deficiency measures. Of the study population, 7.3% were deficient in vitamin B12 (n=397); 37.1% were deficient in vitamin B_6 (n=372); 3.9% were deficient in vitamin A (n=411); 53.9% were deficient or insufficient in 25(OH)-vitamin D (n=410); 9.3% were considered iron depleted based on the soluble transferrin receptor-ferritin index (n=408); and 58.3% were deficient in selenium (n=412) (Table 2).

Demographic risk factors for nutritional deficiency

Risk for established nutritional deficiencies was assessed by demographic and biological factors included in the regression models (race/ethnicity, BMI category, sex, age, tuberculosis disease, hemoglobin level and viral load measured at baseline; results shown in Table 3). For vitamin B_6, white race was significantly associated with deficiency ($p=0.014$). Vitamin A deficiency was associated with lower mean hemoglobin (difference of 2.85 g/dl; $p<0.001$). 25(OH)-vitamin D deficiency was significantly associated with

Asian ethnicity ($p<0.001$) and overweight BMI category ($p=0.042$). Selenium deficiency was associated with black race ($p<0.001$), underweight BMI category (0.042), female sex ($p=0.012$), age under 40 ($p=0.024$), TB disease ($p=0.002$), lower mean hemoglobin levels (difference of 0.69 g/dl; $p<0.001$), and lower mean baseline CD4 count (difference of 17.7 cells/mm^3; $p=0.04$) . High soluble transferrin – ferritin index (indicative of iron deficiency) was significantly associated with overweight BMI category ($p=0.005$), female sex ($p<0.001$), lack of TB disease ($p=0.047$), higher mean baseline CD4 count (difference of 43.6 cells/mm^3; $p=0.003$), lower mean hemoglobin levels (difference of 1.06 g/dl; $p=0.002$), and lower mean viral load (difference of 125542 copies/mL; $p=0.002$).

Linear Analysis of Biomarkers at Week 24

Significant differences were found in CD4 recovery at 24 weeks based on baseline nutritional (Tables 4a, 4b) and inflammatory (Tables 5a, 5b) biomarkers. Univariate analysis (Model A) on nutritional biomarkers revealed statistically significant associations by α-carotene, β-carotene, zeaxanthin, ferritin, the soluble transferrin receptor-ferritin index, and selenium; the inflammatory biomarkers CRP, interferon- γ, IL-6, and IP-10 were also significantly associated in the univariate model. When adjusted for baseline CD4 count (Model B), only zeaxanthin, 25(OH)-vitamin D, and sCD14 were significantly associated with CD4 recovery. After adjustment for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin levels (Model C), α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lycopene, 25(OH)-vitamin D, ferritin, the soluble transferrin receptor-ferritin index, selenium, and IL-6 were significantly associated with CD4 recovery. In the full

combined model, Model D (adjusting for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, baseline hemoglobin levels, and baseline CD4 count), significant associations were found between CD4 recovery and low levels of β-carotene (decrease of 21CD4 cells, 95% CI: 40, 2); low levels of lycopene (decrease of 20 CD4 cells, 95% CI: 39, 2); and 25(OH)-vitamin D deficiency (decrease of 14 CD4 cells, 95% CI: 27, 2).

Linear Analysis of Biomarkers at Week 96

At 96 weeks, we observed similar significant associations between the nutritional (Tables 6a, 6b) and inflammatory (Tables 7a, 7b) biomarkers measured at baseline and the CD4 recovery. Univariate analysis (Model A) revealed statistically significant associations between CD4 recovery and baseline vitamin B_{12}, α-carotene, β-carotene, ferritin, selenium, CRP, and IL-6. When further adjusted for baseline CD4 (Model B), only α-carotene and selenium retained statistically significant associations with overall CD4 recovery. Adjustment for demographic variables only (Model C) revealed significant associations with α-carotene, β-carotene, zeaxanthin, lycopene, 25(OH)-vitamin D, selenium, and IL-6. When using the fully adjusted model (Model D), we observed significant associations between CD4 recovery and lower levels of α-carotene (decrease of 40 CD4 cells, 95% CI: 66, 14); low levels of lycopene (decrease of 28 CD4 cells, 95% CI: 56, 1); selenium deficiency (decrease of 24 CD4 cells, 95% CI: 43, 5); and slightly increased values of soluble CD14 (for quartiles 2 and 3, respectively: increases of 31 CD4 cells, 95% CI: 6, 57 and 31 CD4 cells, 95% CI: 5, 56). We also observed that

25(OH)-vitamin D deficiency was trending significant as well (decrease of 18 CD4 cells) with a p-value of 0.059.

Logistic Analysis of Biomarkers at Week 96

Using the recovery standards previously described, we conducted logistic regression to determine which of the recognized nutritional deficiencies were more likely to affect CD4 recovery at 96 weeks, using the models previously defined for the GLS analyses (Table 8). In all models, vitamin B_{12} deficiency was associated with a higher odds of CD4 non-recovery; in Model D, this association was significant with a p-value of 0.031 (OR=2.00, 95% CI: 1.07, 3.73). Selenium deficiency was also significantly associated with worse CD4 recovery in the fully adjusted model, with a p-value of 0.024 (OR=1.54, 95% CI: 1.06, 2.24).

DISCUSSION

In this analysis we observed micronutrient deficiencies that significantly impacted CD4 recovery to 24 and 96 weeks. In our fully adjusted linear model, selenium deficiency and 25(OH)-vitamin D deficiency were both associated with a poorer CD4 recovery, with decreases at 96 weeks of 24 cells/mm^3 and 18 cells/mm^3, respectively (compared to a non-deficient individual). Lower levels of α-carotene and lycopene were also associated with decreases of 40 cells/mm^3 and 28 cells/mm^3, respectively, at 96 weeks. Our data suggest that baseline nutritional factors, specifically micronutrient status, influence long-term CD4 recovery. Vitamin D, selenium, and carotenoids (including α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, and lycopene) all have antioxidant

properties, and have previously been linked with the inactivation of nuclear factor Kappa B (NF-κB), which plays an important role in the activation of HIV [33].

WHO has long recognized nutrition as an important factor in HIV progression; in its 2003 report on nutritional requirements for people living with HIV/AIDS (PLWH), the impact of nutritional deficiencies on disease progression is explored and further research on this topic is requested [34]. In order to better understand the effect of micronutrients on HIV progression and mortality, recent clinical trials have observed the effect of different types of micronutrient supplementation in HIV-positive individuals [35][36][37][38]. These studies have typically been conducted in a single country and either looking at a single micronutrient such as zinc [38] or a multivitamin supplement which typically includes Vitamins B, C, and E; niacin; and folic acid [35][36][37]. Unsurprisingly, no prospective randomized trials have investigated the effect of micronutrient deficiencies without supplementation in the context of a global cohort that includes both resource-limited and resource-adequate settings [39].

Although micronutrient deficiencies are very common in HIV-infected individuals, the literature on nutritional deficiencies and longitudinal CD4 recovery is relatively limited. Few markers have been independently associated with HIV progression; however, selenium is clearly implicated as having a role in the process [42]. Selenium, like many other micronutrients, has immunomodulatory functions; an irregularity in selenium level may affect a host's susceptibility to infection and the subsequent immune response [43]. Observational studies thus far have been inconclusive but tend to associate lower serum

selenium levels with a higher risk for mortality and opportunistic infections in HIV-infected individuals [44][45]. In the randomized trials that have been conducted thus far, selenium supplementation has also been associated with decreased rate of HIV progression and rates of hospitalization [46][47]. In a 2007 randomized trial conducted by Hurwitz, et al., selenium supplementation was associated with decreased viral load; in a follow-up analysis based on serum selenium levels, high serum selenium was associated with an increase in CD4 count [48]. Results of another randomized trial published in 2013 found that selenium supplementation combined with multivitamin supplementation resulted in a lower risk of significantly decreased CD4 count, AIDS-defining conditions, or AIDS-related death in an asymptomatic ART-naïve cohort of adults [49]. Selenium deficiency has also been associated with a higher risk of mortality from HIV in a cohort of treatment-naïve adults from the United States [24]. However, as of yet there is little information on how selenium deficiency impacts CD4 recovery.

Our findings in regards to selenium, which demonstrate a difference in CD4 recovery based on selenium status, are consistent with our expectations based on the findings from previous observational cohorts and randomized trials. Studies in intravenous drug users including patients both on ART and not on ART have shown that low levels of selenium are associated with HIV-related mortality [24], development of mycobacterial diseases such as tuberculosis [63], and co-infection with HCV [64], all of which have been previously associated with CD4 count. The observational study of pregnant women not on ART in Tanzania showed a positive association between plasma selenium levels and

CD4 count in the first year of follow-up; although our study included only patients on ART, we saw the same relationships [44].

Supplementation with selenium has also been seen to improve outcomes of patients with HIV, including a reduced viral load, indirect improvement of CD4 count, and reduced hospitalizations [46][48]. There are many proposed biological mechanisms related to selenium deficiency in HIV-infected individuals; while this relationship is complicated and multifactorial, the most widely-accepted are gastrointestinal malabsorption of selenium and deficient dietary intake [65][66]. Selenium may not be as well absorbed during the normal digestion process, which can lead to lower levels of selenium entering the bloodstream. While the relationship is not confirmed due to lack of adequate statistical studies, there does seem to be a relationship between selenium uptake in the gastrointestinal tract and HIV infection independent of gastrointestinal issues such as diarrhea, bowel pathogens, or general gastrointestinal malabsorption [65]. Selenium deficiency can also lead to impaired immune function, but supplementation has shown to restore immune activity in deficient individuals [49]. It is likely that the antiviral and antioxidant properties of selenium seen *in vitro* have an effect on HIV; due to these properties, when an individual is deficient in selenium HIV will actively replicate at a higher rate, thus destroying more CD4 cells and causing a lowered CD4 recovery compared to a non-deficient individual [23].

Deficiency of 25(OH)-vitamin D, has also been shown to affect HIV progression in treatment-naïve patients. In the ANRA-C09-COPANA cohort, a prospective study of

newly diagnosed HIV-1 infected individuals being conducted in 37 hospitals in France, patients classified as 25(OH)-vitamin D deficient at baseline were also more likely to have a CD4 count of <100 cells/mm^3 [50]. However, this association was reduced once the analysis was adjusted for inflammatory markers such as hsCRP, sTNFR1, and sTNFR2; this implies that the association can be partly attributed to the actions of these inflammatory markers [50]. A similar finding was also replicated in a study from the Women's Interagency HIV Study (WIHS); HIV-positive women who were 25(OH)-vitamin D insufficient or deficient were more likely to have a diminished CD4 recovery after HAART initiation to 24 months [51]. In another prospective study, 25(OH)-vitamin D was found to be positively associated with improved immune restoration after initiating ART [52].

However, the evidence for the association of 25(OH)-vitamin D and CD4 recovery is not as strong from a supplementation standpoint. A 2011 study conducted in HIV-infected children in Toronto (75% of whom were 25(OH)-vitamin D insufficient or deficient) observed no increase in CD4 count compared to a placebo after a six-month regimen of high-level 25(OH)-vitamin D supplementation [53]. Despite the high prevalence of 25(OH)-vitamin D deficiency in HIV-infected adults, the exact relationship between 25(OH)-vitamin D and CD4 recovery remains unclear [54].

Our analysis found that baseline 25(OH)-vitamin D levels are associated with worse long-term CD4 recovery, which is generally supported by the literature; both cross-sectional and supplementation studies indicate that a deficiency of 25(OH)-vitamin D

among HIV-infected individuals can lead to more adverse outcomes such as mortality and progression to AIDS [67]. However, one study of 25(OH)-vitamin D supplementation in children showed no increase in CD4 cell count with a high level of 25(OH)-vitamin D supplementation, and not all of the treated children even reached sufficiency [53]. While we did not see a similar relationship in this study, the children in the supplementation study entered with relatively high CD4 counts (mean of 927 cells/mm^2) compared to our study population, which only included individuals with a CD4 count of less than 350 cells/mm^3 at baseline [53]. However, this raises an interesting point – if 25(OH)-vitamin D supplementation is not very effective in boosting CD4 recovery for individuals found to be deficient before ART initiation, should we test at all? The potential benefits need to be explored and weighed with the cost and difficulty of these tests in order to make decisions that will have a meaningful impact for patients. More research is needed to determine the specific effect of 25(OH)-vitamin D deficiency on CD4 recovery after initiation of ART.

Iron status has also been implicated in the progression of HIV, although the literature thus far has been inconclusive. It has been proposed that high iron stores may have an adverse effect on immunity and the function of the immune system; retrospective studies in the serum and bone marrow have observed that the rate of HIV progression was significantly faster in patients with higher iron status (measured by serum ferritin concentration and iron stores in bone marrow aspirations) [55]. However, other studies in both adults and children have indicated that iron-deficiency anemia independently predicts adverse outcomes such as more severe clinical presentation and more severe

immunosuppression [56][57][58][59]. Yet other studies have found that iron stores measured by serum levels of soluble transferrin receptor and ferritin are not related to HIV progression, although they are indicative of anemia [60]. Additionally, the relationship between HIV progression and iron status is complicated by the fact that iron status is also associated with common HIV co-morbidities such as tuberculosis and malaria [61][62]. Our findings show a moderately strong relationship between iron deficiency and CD4 count at week 24 in the GLS models when measured by the soluble transferrin receptor – ferritin index (CD4 count decreases by approximately 40 cells/mm^3), although the relationship becomes non-significant when the model is adjusted for baseline CD4 count. While this is the general relationship we were expecting to see, further investigation is necessary to determine how baseline CD4 count and iron stores and use are associated in the context of HIV infection.

In our findings we also saw relationships between CD4 recovery and carotenoids, most notably the association observed between CD4 recovery and levels of β-carotene. Carotenoids (including α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, and lycopene) have antioxidant properties and lower levels of carotenoids have been associated with higher rates of HIV seroconversion and HIV-related symptoms [70][71]. In our study, low levels of β-carotene were significantly associated with worse CD4 recovery at both 24 weeks and 96 weeks in the GLS models. Previously, β-carotene has been seen in lower levels in treatment-naïve HIV-infected children than in HIV-uninfected children, which was hypothesized to be a consequence of β-carotene's elimination of free radicals under normal conditions [71]. When an individual is deficient

in β-carotene, free radicals are able to enhance the activation of NF-κB, which is known to promote HIV replication [71]. Because these deficiencies can allow for greater activation and replication of HIV, more viruses are able to infect and destroy CD4 cells, thus leading to a decrease in CD4 cells as the disease progresses. Thus, it is not surprising that most micronutrient supplementation studies include carotenoids (most commonly, α-carotene and β-carotene) in supplementation regimens. In countries like South Africa, where we observed that approximately one-third of the study population was in the lowest quartile of β-carotene levels, it may be feasible to start all treatment naïve HIV patients on a micronutrient supplementation regimen that includes β-carotene as a way to boost immune function and prevent further adverse events.

Vitamin B_{12} was also strongly implicated in the logistic regression, although only to a small extent in the GLS analysis. In all GEE models, vitamin B_{12} deficiency essentially doubled the odds of not achieving CD4 recovery by 96 weeks based on the thresholds at 24, 48, and 96 weeks. Previously, an association has been seen between low levels of vitamin B_{12} and HIV disease; it has been seen in multiple countries that vitamin B_{12} levels are generally lower in HIV patients [72][73]. Similar to β-carotene, malabsorption is thought to be a mechanism through which vitamin B_{12} deficiency is mediated through HIV infection [74][75]. One retrospective study in Uganda found that sub-optimal levels of vitamin B_{12} were associated with a longer known duration of HIV infection and higher rate of CD4 decline pre-ART; the authors suspect that the observed deficiency was due to an increased demand to replace CD4 cells depleted by HIV, although they did not have data to support that theory [73]. Supplementation studies have likewise shown little about

the effects of vitamin B_{12} alone – because many supplementation studies include vitamin B_{12} as part of a complex with vitamin B_6 or as part of a multivitamin with many components, it is difficult to parse out the independent effect of vitamin B_{12} on HIV-related outcomes. More investigation with prospective and molecular studies are needed in order to better understand this relationship.

Inflammatory and immune activation biomarkers have also been associated with HIV and disease progression, as an increase in these markers signals that the immune system is taking action against the virus. However, the exact biological role of these markers in HIV infection and CD4 recovery is not known. Previous studies have shown that increased plasma levels of sCD14, a marker of monocyte activation, were associated with two markers of HIV disease progression, hsCRP and D-dimer [40]. Increased levels of sCD14 are also the only known strong independent predictor of mortality in HIV-infected individuals among immune activation markers [27][41]. In our study, sCD14 was the only immune activation or inflammatory marker found to be significantly associated with CD4 recovery in the linear models; however, at 96 weeks, moderately increased levels (quartiles 2 and 3) of sCD14 were associated with a higher CD4 count compared to the lowest quartile. No other studies have investigated this relationship, so it is possible that sCD14 is working to increase markers of HIV progression via a pathway other than CD4 cells. More investigation is needed to more fully understand this relationship and how sCD14 and other markers of immune activation could be potentially targeted for clinical purposes.

We also observed an interesting result in the demographics of the groups who were deficient in nutritional biomarkers. Because most of the deficiencies measured here had unique demographic risk factors (including sex, age, country, race, BMI category, and TB at baseline), it is difficult to create a risk factor profile that is applicable across nutrients and locations. We were not able to observe any demographic risk factor that was a risk factor for deficiency across all nutrients; there was even very little consistency in deficiencies across countries. For instance, Brazil, which generally ranked low to average among deficient countries, had the highest rate of selenium deficiency – a micronutrient for which low levels were seen in Haiti and the United States. Because there are such great differences in risk factors for each micronutrient and between the countries, it is imperative that researchers and clinicians understand the context in which they are working, and look for these risk factors on a more local level.

An important consideration for this cohort was finding an appropriate time point at which to look for associations between biomarkers and CD4 recovery. In the past, it has been shown that other markers such as weight were relatively reliable indicators of individuals at high risk for adverse outcomes at times as early as one month after treatment initiation; however, because our primary outcome was CD4 recovery, we had to take into account the biological processes occurring surrounding CD4 cells after treatment initiation [68]. In approximately the first eight weeks after treatment initiation, the increase in circulating CD4 cells can be attributed mainly to migration of the CD4 cells from the lymphoid tissues to blood [69]. Because these cells are not newly-created lymphocytes, this initial short-term (8-12 weeks) increase seen in the blood is not necessarily indicative of the

revival of either short- or long-term immune response due to treatment. In fact, the intensity of this initial recovery in CD4 cells is closely associated with the overall relative depletion of CD4 cells at the time of treatment initiation, as well as the slope of the CD4 decline in the year prior to treatment [69]. Because of this lag in indication of CD4 cells in blood, we chose to use 24 weeks as a marker of our short-term CD4 recovery. In this study, CD4 counts and other data were collected until week 96, which is the timepoint we used to observe long-term CD4 recovery; however, recovery is typically observed clinically over five or more years in order to preclude immunologic failure [20]. Yet we saw many of the same associations between CD4 recovery and biomarkers in both the short- and long-term analyses; this may indicate that once treatment is initiated, there is little change in trajectory over time.

These findings have several limitations. For cost and logistic reasons, measurement of the biomarkers in the PEARLS cohort was only undertaken for a smaller cohort of participants instead of the full study cohort, and we could not include the numerous individuals in the analysis for which there was no data. There were also very few cases of immunologic failure in the entire cohort (n=14), which affected our ability to investigate factors associated with immunologic failure. This may indicate that our cohort is generally healthier than the population of individuals starting ART treatment in these countries, which may bias our study and limit the generalizability of our results. For micronutrients in which only a small percentage of the population was considered deficient (such as vitamin B_{12}), it was difficult to see an effect on CD4 recovery with such a small sample size, which may be contributing to the lack of effect we observed.

Missing data is also an issue for some of the samples (specifically from India), as there were delays in processing and customs that caused the samples to be lost; this has the potential to affect our conclusions if the participants in India were different than the participants in the rest of the cohort.

Despite these limitations, this analysis demonstrates the global importance of micronutrient deficiencies on HIV progression and CD4 recovery in treatment-naïve patients. Although there was a large amount of heterogeneity in the cohort due to the nine study locations, the findings were still discriminatory; this lends power to the study and validates the findings on a global scale. With participants in nine low-, intermediate-, and high-income countries on four continents, this is a unique and globally representative cohort. Standardization of study protocols, enrollment procedures and criteria, lab analysis techniques, and quality assurance/control measures ensured that the data was of high quality and consistent across study sites. Moving forward with this research, we should continue to focus on comprehensive high-quality data collection in a diverse global context.

In conclusion, lower baseline levels of micronutrients (specifically, selenium and 25(OH)-vitamin D) in HIV-infected patients at ART initiation are associated with worse CD4 recovery in both the short- and the long-term. These findings suggest that in the future, baseline levels of these micronutrients could be a valuable tool for clinical decision-making and providing supplementary treatment for HIV-infected patients who are at increased risk of diminished CD4 recovery.

Table 1. Characteristics of the study population (n=469)	
Gender	
Male	254 (54.2)
Female	215 (45.8)
Median Age (IQR)	35 (29-40)
BMI (kg/m^2)	
<18.5	58 (12.4)
18.5 - 25	315 (67.2)
>25	96 (20.5)
Screening CD4 (cells/mm^3)	153.2 (84.6)
Race	
White	26 (5.5)
Black	222 (47.3)
Hispanic	75 (16.0)
Asian	146 (31.1)
Country	
Brazil	45 (9.6)
Haiti	38 (8.1)
India	107 (22.8)
Malawi	62 (13.2)
Peru	36 (7.7)
South Africa	58 (12.4)
Thailand	37 (7.9)
United States	48 (10.2)
Zimbabwe	38 (8.1)
TB at baseline	112 (23.4)
Hgb at baseline (g/dl)*	12.1 (2.0)
VL at baseline (cells/mL)†	218192.6 (229007.9)

Data are presented as n (%) unless otherwise specified. P-values were calculated from t-tests and Fisher's exact test for continous and categorical variables, respectively

* 465 individuals included in analysis due to missing samples

† 468 individuals included in analysis due to missing samples

Table 2. Baseline prevalence of nutritional deficiency measures by country						
	Vitamin B12 (n = 397)	**Vitamin B6 (n = 372)**	**Vitamin A (n = 411)**	**25(OH)-vitamin D (n = 410)**	**Selenium (n = 412)**	**Transferrin Receptor-Ferritin Index (n = 408)**
Brazil	3 (6.7)	18 (40.9)	0 (0)	12 (26.7)	41 (91.1)	1 (2.2)
Haiti	5 (13.5)	13 (35.1)	2 (5.4)	12 (32.4)	2 (5.4)	2 (5.4)
India	4 (6.7)	12 (21.8)	1 (1.6)	45 (72.6)	28 (45.2)	2 (3.2)
Malawi	0 (0)	27 (48.2)	6 (9.8)	24 (40.7)	57 (93.4)	10 (17.0)
Peru	3 (8.6)	10 (50.0)	1 (2.9)	15 (42.9)	12 (34.3)	3 (8.9)
South Africa	2 (4.3)	15 (30.0)	2 (3.7)	32 (58.2)	48 (87.3)	7 (13.0)
Thailand	4 (12.9)	7 (26.9)	0 (0)	24 (75.0)	14 (43.8)	3 (9.4)
United States	1 (2.3)	19 (40.4)	0 (0)	38 (79.2)	1 (2.1)	6 (12.5)
Zimbabwe	7 (18.9)	17 (46.0)	4 (10.8)	19 (51.4)	37 (100)	4 (11.1)
TOTAL	**29 (7.3)**	**138 (37.1)**	**16 (3.9)**	**221 (53.9)**	**240 (58.3)**	**38 (9.3)**

Data presented as No. (% deficiency by country)

Table 3. Baseline demographic and biological risk factors for nutritional deficiencies

	Vitamin B12 Deficiency (n = 29)	Vitamin B6 Deficiency (n = 138)	Vitamin A Deficiency (n = 16)	25(OH)-vitamin D Deficiency (n = 221)	Selenium Deficiency (n = 240)	Transferrin Receptor-Ferritin Index Deficiency (n = 38)
Gender						
Male	20 (9.4)	73 (37.1)	5 (2.2)	117 (52.0)	119 (52.3)	7 (3.1)
Female	9 (4.9)	65 (37.1)	11 (5.9)	104 (56.2)	**121 (65.1)***	**31 (17.0)***
Age						
<40	15 (5.5)	88 (35.8)	11 (3.9)	151 (53.9)	**174 (62.1)***	25 (9.1)
>40	14 (11.1)	50 (39.68)	5 (3.8)	70 (53.9)	66 (50.0)	13 (9.9)
BMI (kg/m^2)						
<18.5	0 (0)	17 (40.5)	3 (6.7)	26 (57.8)	**31 (68.9)***	4 (8.9)
18.5 - 25	21 (7.8)	91 (36.6)	12 (4.3)	138 (49.8)	167 (59.9)	18 (6.5)
>25	8 (9.4)	30 (37.0)	1 (1.1)	**57 (64.8)***	42 (47.7)	**16 (18.8)***
Race						
White	1 (4)	**12 (46.2)***	0 (0)	12 (46.2)	15 (57.7)	0 (0)
Black	14 (6.8)	86 (42.0)	14 (6.5)	110 (51.4)	**148 (68.5)***	**27 (12.7)***
Hispanic	6 (8.1)	21 (36.2)	1 (1.4)	28 (37.8)	34 (46.0)	6 (8.1)
Asian	8 (8.6)	19 (22.9)	1 (1.0)	**71 (74.0)****	43 (44.8)	5 (5.2)
TB at Screening						
No TB	21 (6.9)	109 (38.7)	12 (3.8)	166 (52.9)	171 (54.1)	**34 (10.9)***
TB	8 (8.7)	29 (32.2)	4 (4.2)	55 (57.3)	**69 (71.9)***	4 (4.2)
Mean difference in baseline CD4 (cells/mm^3)†	20.76	14.6	4.43	0.27	**17.7***	**-43.6***
Mean difference in Hgb at Screening (g/dl)†	-0.07	0.3	**2.9****	0.22	**0.69****	**1.06****
Mean difference in VL at Screening (cells/mL)†	-28481	12263	68319	36589	-29452	**125542****

Data are presented as n (%) unless otherwise specified.
P-values were calculated from t-tests and Fisher's exact test for continous and categorical variables, respectively
† Mean difference = (mean in non-deficient individuals) - (mean in deficient individuals)
* p<0.5; ** p<0.001

Table 4a. Nutritional biomarkers associated with CD4 recovery at 24 weeks (A & B)*						
	MODEL A			MODEL B		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
α-tocopherol		REF			REF	
Q3	10.59	0.494	[-19.76, 40.94]	0.38	0.967	[-17.47, 18.22]
Q2	16.62	0.283	[-13.73, 46.98]	-9.59	0.295	[-27.54, 8.36]
Q1	6.42	0.679	[-24.01, 36.85]	-2.07	0.821	[-19.98, 15.84]
Vitamin B12	-39.47	0.065	[-81.41, 2.47]	-18.07	0.151	[-42.72, 6.57]
Vitamin B6	-14.62	0.222	[-38.09, 8.86]	1.03	0.880	[-12.35, 14.41]
Retinol	-0.44	0.988	[-56.12, 55.25]	4.55	0.787	[-28.39, 37.49]
γ-tocopherol		REF			REF	
Q3	1.68	0.913	[-28.67, 32.04]	-2.57	0.777	[-20.39, 15.24]
Q2	16.28	0.293	[-14.07, 46.62]	10.27	0.260	[-7.60, 28.13]
Q1	14.64	0.344	[-15.68, 44.95]	2.20	0.809	[-15.64, 20.05]
α-carotene		REF			REF	
Q3	3.19	0.824	[-24.98, 31.36]	-1.56	0.855	[-18.27, 15.16]
Q2	-27.78	0.074	[-58.23, 2.66]	-9.78	0.290	[-27.88, 8.33]
Q1	**-38.37**	**0.018**	**[-70.12, -6.63]**	-13.81	0.153	[-32.76, 5.14]
β-carotene		REF			REF	
Q3	**-30.50**	**0.04**	**[-59.54, -1.46]**	4.08	0.648	[-13.45, 21.61]
Q2	**-46.21**	**0.002**	**[-75.94, -16.48]**	-14.07	0.124	[-31.99, 3.84]
Q1	**-66.02**	**<0.001**	**[-96.29, -35.74]**	-12.74	0.178	[-31.28, 5.80]
β-cryptoxanthin		REF			REF	
Q3	23.89	0.117	[-5.97, 53.76]	0.62	0.946	[-17.13, 18.37]
Q2	13.83	0.354	[-15.41, 43.07]	2.26	0.799	[-15.10, 19.62]
Q1	-15.33	0.331	[-46.26, 15.60]	4.15	0.658	[-14.24, 22.54]
Lutein		REF			REF	
Q3	8.78	0.557	[-20.54, 38.10]	2.58	0.771	[-14.76, 19.92]
Q2	12.68	0.415	[-17.78, 43.14]	14.52	0.114	[-3.47, 32.50]
Q1	-27.85	0.066	[-57.53, 1.83]	0.69	0.939	[-16.99, 18.38]
Zeaxanthin		REF			REF	
Q3	**27.11**	**0.046**	**[0.523, 53.69]**	**21.17**	**0.008**	**[5.48, 36.87]**
Q2	2.80	0.861	[-28.41, 34.0]	17.20	0.068	[-1.27, 35.67]
Q1	-27.63	0.092	[-59.82, 4.55]	6.15	0.530	[-13.06, 25.37]
Lycopene		REF			REF	
Q3	-18.69	0.223	[-48.73, 11.35]	1.91	0.833	[-15.86, 19.67]
Q2	-9.52	0.536	[-39.69, 20.66]	2.35	0.797	[-15.50, 20.19]
Q1	-30.94	0.047	[-61.52, -0.37]	-5.70	0.539	[-23.87, 12.47]
25-OH Vitamin D	-16.71	0.129	[-38.26, 4.84]	**-16.59**	**0.010**	**[-29.17, -4.00]**
Transferrin: Ferritin Index	**43.11**	**0.022**	**[6.27, 79.94]**	-2.36	0.833	[-24.34, 19.61]
Selenium	**-23.12**	**0.036**	**[-44.79, -1.46]**	-4.92	0.453	[-17.76, 7.93]

*Models are defined as follows:

Model A: Univariate

Model B: Adjusted for screening CD4

Table 4b. Nutritional biomarkers associated with CD4 recovery at 24 weeks (C & D)*						
	MODEL C			MODEL D		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
α-tocopherol		REF			REF	
Q3	19.94	0.194	[-10.17, 50.04]	-1.73	0.844	[-18.95, 15.48]
Q2	17.70	0.251	[-12.50, 47.90]	-7.65	0.386	[-24.96, 9.66]
Q1	13.96	0.396	[-18.25, 46.18]	-2.14	0.819	[-20.54, 16.25]
Vitamin B12	-27.08	0.202	[-68.63, 14.48]	-16.25	0.176	[-39.77, 7.27]
Vitamin B6	-6.83	0.565	[-30.12, 16.46]	4.76	0.472	[-8.21, 17.73]
Retinol	30.18	0.295	[-26.33, 86.68]	4.23	0.798	[-28.17, 36.64]
γ-tocopherol		REF			REF	
Q3	3.01	0.849	[-27.89, 33.91]	-0.11	0.990	[-17.60, 17.37]
Q2	11.28	0.491	[-20.78, 43.34]	12.11	0.192	[-6.07, 30.29]
Q1	5.51	0.755	[-29.06, 40.08]	-2.26	0.821	[-21.86, 17.34]
α-carotene		REF			REF	
Q3	-0.43	0.976	[-28.82, 27.96]	-7.63	0.356	[-23.85, 8.59]
Q2	-25.74	0.101	[-56.52, 5.05]	-14.80	0.099	[-32.38, 2.78]
Q1	**-33.15**	**0.044**	**[-65.48, -0.82]**	-17.77	0.060	[-36.30, 0.76]
β-carotene		REF			REF	
Q3	-22.64	0.131	[-52.05, 6.77]	-4.37	0.611	[-21.24, 12.49]
Q2	**-34.12**	**0.029**	**[-64.80, -3.44]**	-17.47	0.051	[-35.04, 0.10]
Q1	**-53.58**	**0.001**	**[-86.03, -21.12]**	**-20.92**	**0.028**	**[-39.63, -2.20]**
β-cryptoxanthin		REF			REF	
Q3	**31.59**	**0.037**	**[1.94, 61.24]**	2.44	0.779	[-14.61, 19.49]
Q2	19.24	0.211	[-10.92, 49.39]	0.05	0.996	[-17.28, 17.38]
Q1	8.56	0.611	[-24.46, 41.58]	3.09	0.749	[-15.80, 21.98]
Lutein		REF			REF	
Q3	7.66	0.614	[-22.07, 37.38]	-6.33	0.465	[-23.30, 10.65]
Q2	19.63	0.236	[-12.81, 52.08]	7.69	0.415	[-10.79, 26.18]
Q1	-23.35	0.156	[-55.64, 8.93]	-16.22	0.085	[-34.65, 2.22]
Zeaxanthin		REF			REF	
Q3	**39.05**	**0.004**	**[12.28, 65.81]**	15.16	0.054	[-0.29, 30.60]
Q2	16.24	0.316	[-15.48, 47.96]	0.58	0.950	[-17.67, 18.83]
Q1	-5.44	0.751	[-39.07, 28.18]	-3.70	0.708	[-23.06, 15.66]
Lycopene		REF			REF	
Q3	-22.96	0.139	[-53.34, 7.43]	-4.83	0.584	[-22.29, 30.69]
Q2	-15.95	0.318	[-47.24, 15.33]	-6.62	0.467	[-24.46, 11.21]
Q1	**-38.05**	**0.023**	**[-70.85, -5.25]**	**-20.35**	**0.033**	**[-39.10, -1.60]**
25-OH Vitamin D	**-22.78**	**0.042**	**[-44.71, -0.84]**	**-14.07**	**0.027**	**[-26.55, -1.60]**
Transferrin: Ferritin Index	**42.24**	**0.030**	**[4.10, 80.38]**	1.69	0.880	[-20.25, 23.63]
Selenium	**-23.80**	**0.036**	**[-46.08, -1.52]**	-9.18	0.158	[-21.92, 3.56]

*Models are defined as follows:

Model C: Adjusted for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin

Model D: Model C + adjustment for screening CD4

Table 5a. Inflammatory biomarkers associated with CD4 recovery at 24 weeks (A & B)*						
	MODEL A			MODEL B		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
CRP		REF			REF	
Q2	3.12	0.837	[-26.70, 32.94]	2.49	0.784	[-15.32, 20.29]
Q3	**-35.57**	**0.019**	**[-65.38, -5.75]**	-7.91	0.388	[-25.86, 10.05]
Q4	**-49.96**	**0.001**	**[-79.69, -20.23]**	-10.16	0.269	[-28.19, 7.86]
LPS	**-24.06**	**0.023**	**[-44.74, -3.39]**	-0.19	0.977	[-12.83, 12.46]
sCD14		REF			REF	
Q2	21.95	0.162	[-8.83, 52.72]	12.55	0.171	[-5.42, 30.51]
Q3	15.94	0.311	[-14.93, 46.81]	11.27	0.221	[-6.76, 29.30]
Q4	-4.11	0.789	[-34.24, 26.02]	**17.80**	**0.048**	**[-0.12, 35.49]**
Endocab		REF			REF	
Q2	3.70	0.810	[-26.51, 33.91]	-0.45	0.960	[-18.23, 17.33]
Q3	1.90	0.902	[-28.27, 32.07]	3.30	0.716	[-14.44, 21.04]
Q4	1.13	0.942	[-29.07, 31.32]	1.38	0.879	[-16.38, 19.14]
Interferon-γ		REF			REF	
Q2	-10.92	0.494	[-42.24, 20.40]	4.52	0.629	[-13.80, 22.84]
Q3	**-32.29**	**0.044**	**[-63.74, -0.84]**	-7.33	0.437	[-25.81, 11.16]
Q4	**-51.94**	**0.001**	**[-83.33, -20.54]**	-6.29	0.509	[-24.95, 12.38]
IL-6		REF			REF	
Q2	**-32.48**	**0.042**	**[-63.79, -1.17]**	-2.03	0.829	[-20.51,16.44]
Q3	**-59.39**	**<0.001**	**[-90.58, -28.21]**	-10.16	0.285	[-28.80, 8.47]
Q4	**-39.77**	**0.013**	**[-71.0, -8.54]**	-0.589	0.950	[-19.15, 17.97]
IP-10		REF			REF	
Q2	-18.42	0.254	[-50.04, 13.20]	-6.1	0.514	[-24.40, 12.20]
Q3	-21.43	0.186	[-53.20, 10.34]	-5.61	0.550	[-24.0, 12.78]
Q4	**-33.90**	**0.036**	**[-65.51, -2.27]**	5.84	0.537	[-12.68, 24.35]
TNF-α		REF			REF	
Q2	3.80	0.815	[-28.07, 33.67]	16.18	0.084	[-2.16, 34.51]
Q3	1.37	0.933	[-30.58, 33.32]	8.38	0.371	[-9.99, 26.76]
Q4	-12.28	0.450	[-44.15, 19.59]	14.32	0.128	[-4.12, 32.76]

*Models are defined as follows:

Model A: Univariate

Model B: Adjusted for screening CD4

Table 5b. Inflammatory biomarkers associated with CD4 recovery at 24 weeks (C & D)*						
	MODEL C			MODEL D		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
CRP		REF			REF	
Q2	6.14	0.684	[-23.44, 35.72]	0.792	0.927	[-16.06, 17.64]
Q3	-18.47	0.231	[-48.70, 11.76]	-5.88	0.505	[-23.17, 11.41]
Q4	-26.18	0.104	[-57.71, 5.34]	-12.33	0.180	[-30.33, 5.68]
LPS	-17.43	0.104	[-38.46, 3.61]	6.78	0.283	[-5.60, 19.13]
sCD14		REF			REF	
Q2	14.65	0.342	[-15.58, 44.88]	16.88	0.052	[-0.178, 33.94]
Q3	13.32	0.396	[-17.43, 44.06]	13.65	0.123	[-3.71, 31.02]
Q4	6.62	0.676	[-24.43, 37.68]	14.04	0.118	[-3.55, 31.64]
Endocab		REF			REF	
Q2	-5.97	0.695	[-35.81, 23.86]	-4.42	0.611	[-21.42, 12.59]
Q3	-0.21	0.989	[-29.96, 29.54]	-0.23	0.978	[-17.15, 16.68]
Q4	-0.72	0.963	[-30.76, 29.32]	-5.81	0.506	[-22.91, 11.30]
Interferon-γ		REF			REF	
Q2	-5.23	0.744	[-36.60, 26.14]	2.47	0.783	[-15.09, 20.02]
Q3	-24.33	0.129	[-55.73, 7.07]	-10.74	0.232	[-28.36, 6.88]
Q4	-39.38	0.014	[-70.64, -8.13]	-6.75	0.454	[-24.42, 10.91]
IL-6		REF			REF	
Q2	-25.43	0.112	[-56.74, 5.89]	-6.61	0.461	[-24.20, 10.98]
Q3	**-51.36**	**0.001**	**[-83.03, -19.68]**	-16.97	0.063	[-34.89, 0.95]
Q4	-28.14	0.079	[-59.54, 3.26]	-4.12	0.649	[-21.82, 13.59]
IP-10		REF			REF	
Q2	-18.18	0.254	[-49.41, 13.06]	-3.21	0.718	[-20.61, 14.19]
Q3	-18.08	0.264	[-49.82, 13.67]	-9.96	0.269	[-27.61, 7.70]
Q4	-26.78	0.110	[-59.62, 6.07]	-13.10	0.161	[-31.43, 5.23]
TNF-α		REF			REF	
Q2	7.38	0.647	[-24.23, 39.0]	8.97	0.317	[-8.61, 26.55]
Q3	13.77	0.398	[-18.14, 45.68]	-1.25	0.890	[-19.03, 16.52]
Q4	3.38	0.841	[-29.69, 36.44]	-2.83	0.763	[-21.23, 15.58]

*Models are defined as follows:

Model C: Adjusted for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin

Model D: Model C + adjustment for screening CD4

Table 6a. Nutritional biomarkers associated with CD4 recovery at 96 weeks (A & B)*						
	MODEL A			MODEL B		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
α-tocopherol		REF			REF	
Q3	5.63	0.759	[-30.4, 41.66]	-4.72	0.724	[-30.97, 21.53]
Q2	11.06	0.547	[-24.97, 47.09]	-15.05	0.263	[-41.42, 11.33]
Q1	-9.42	0.61	[-45.58, 26.75]	-17.71	0.188	[-44.09, 8.67]
Vitamin B12	**-51.94**	**0.041**	**[-101.68, -2.21]**	-30.52	0.099	[-66.73, 5.69]
Vitamin B6	-26.36	0.063	[-54.17, 1.46]	-10.66	0.294	[-30.58, 9.25]
Retinol	-19.28	0.568	[-85.56, 47.0]	-13.96	0.573	[-62.48, 34.56]
γ-tocopherol		REF			REF	
Q3	5.72	0.755	[-30.28, 41.73]	1.10	0.935	[-25.15, 27.35]
Q2	16.37	0.373	[-19.67, 52.4]	9.75	0.468	[-16.57, 36.08]
Q1	26.84	0.144	[-9.15, 62.83]	14.31	0.286	[-12.0, 40.61]
α-carotene		REF			REF	
Q3	4.24	0.803	[-29.06, 37.53]	-0.73	0.953	[-25.17, 23.70]
Q2	**-46.68**	**0.011**	**[-82.72, -10.64]**	**-29.09**	**0.032**	**[-55.62, -2.56]**
Q1	**-40.03**	**0.037**	**[-77.62, -2.43]**	-15.11	0.286	[-42.87, 12.66]
β-carotene		REF			REF	
Q3	-15.67	0.376	[-50.33, 18.99]	18.85	0.151	[-6.85, 44.56]
Q2	**-47.26**	**0.009**	**[-82.76, -11.77]**	-15.02	0.263	[-41.32, 11.27]
Q1	**-61.50**	**0.001**	**[-97.67, -25.32]**	-7.43	0.592	[-34.63, 19.76]
β-cryptoxanthin		REF			REF	
Q3	17.21	0.342	[-18.26, 52.68]	-6.42	0.630	[-32.52, 19.69]
Q2	2.08	0.907	[-32.65, 36.81]	-9.37	0.471	[-34.88, 16.14]
Q1	-29.11	0.12	[-65.86, 7.63]	-9.60	0.486	[-36.63, 17.42]
Lutein		REF			REF	
Q3	8.14	0.648	[-26.79, 43.07]	1.78	0.891	[-23.75, 27.32]
Q2	17.06	0.356	[-19.19, 53.32]	18.96	0.160	[-7.50, 45.43]
Q1	-22.71	0.208	[-58.09, 12.67]	6.31	0.635	[-19.74, 32.35]
Zeaxanthin		REF			REF	
Q3	23.00	0.154	[-8.61, 54.63]	16.52	0.163	[-6.71, 39.75]
Q2	4.57	0.809	[-32.58, 41.73]	19.09	0.171	[-8.26, 46.44]
Q1	-37.98	0.052	[-76.36, 0.394]	-4.24	0.771	[-32.72, 24.24]
Lycopene		REF			REF	
Q3	-9.71	0.594	[-45.45, 26.02]	10.68	0.423	[-15.45, 36.80]
Q2	-9.25	0.614	[-45.18, 26.68]	2.44	0.855	[-23.82, 28.69]
Q1	-26.68	0.151	[-63.04, 9.71]	-1.27	0.925	[-27.95, 25.41]
25-OH Vitamin D	-16.44	0.208	[-42.02, 9.15]	-16.29	0.086	[-34.88, 2.31]
Transferrin: Ferritin Index	42.98	0.054	[-0.78, 86.74]	-2.50	0.880	[-34.78, 29.78]
Selenium	**-39.33**	**0.003**	**[-64.88, -13.77]**	**-21.30**	**0.026**	**[-40.08, -2.51]**

*Models are defined as follows:

Model A: Univariate

Model B: Adjusted for screening CD4

Table 6b. Nutritional biomarkers associated with CD4 recovery at 96 weeks (C & D)*						
	MODEL C			MODEL D		
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
α-tocopherol		REF			REF	
Q3	17.26	0.341	[-18.27, 52.80]	-4.72	0.718	[-30.29, 20.85]
Q2	14.98	0.410	[-20.67, 50.64]	-10.18	0.437	[-35.87, 15.51]
Q1	1.86	0.924	[-36.22, 39.93]	-13.93	0.318	[-41.30, 13.44]
Vitamin B12	-38.19	0.125	[-87.03, 10.65]	-27.53	0.121	[-62.33, 7.27]
Vitamin B6	-15.29	0.276	[-42.80, 12.22]	-3.87	0.698	[-23.37, 15.64]
Retinol	34.11	0.632	[-50.52, 83.20]	-8.98	0.715	[-57.12, 39.16]
γ-tocopherol		REF			REF	
Q3	6.96	0.708	[-29.50, 43.42]	3.60	0.787	[-22.49, 29.70]
Q2	8.20	0.671	[-29.68, 46.07]	8.49	0.540	[-18.67, 35.64]
Q1	10.93	0.600	[-29.88, 51.74]	3.18	0.831	[-26.07, 32.43]
α-carotene		REF			REF	
Q3	-4.17	0.806	[-37.45, 29.10]	-11.72	0.336	[-35.61, 12.16]
Q2	**-50.31**	**0.006**	**[-86.44,-14.18]**	**-39.68**	**0.003**	**[-65.63, -13.73]**
Q1	**-38.52**	**0.047**	**[-76.49, -0.55]**	-22.80	0.102	[-50.15, 4.55]
β-carotene		REF			REF	
Q3	-6.82	0.701	[-41.64, 27.99]	11.34	0.375	[-13.70, 36.39]
Q2	-31.66	0.088	[-67.99, 4.66]	-15.13	0.256	[-41.25, 10.98]
Q1	**-45.12**	**0.021**	**[-83.57, -6.68]**	-12.01	0.397	[-39.83, 15.80]
β-cryptoxanthin		REF			REF	
Q3	27.57	0.123	[-7.47, 62.61]	-1.81	0.889	[-27.13, 23.51]
Q2	7.07	0.697	[-28.58, 42.73]	-11.92	0.364	[-37.63, 13.79]
Q1	-1.90	0.924	[-40.91, 37.11]	-7.26	0.611	[-35.27, 20.75]
Lutein		REF			REF	
Q3	5.24	0.770	[-29.89, 40.37]	-8.71	0.499	[-33.98, 16.57]
Q2	18.51	0.344	[-19.82, 56.84]	6.56	0.640	[-20.96, 34.08]
Q1	-25.41	0.192	[-63.60, 12.77]	-17.81	0.204	[-45.27, 9.66]
Zeaxanthin		REF			REF	
Q3	**37.46**	**0.020**	**[5.81, 69.11]**	13.33	0.256	[-9.65, 36.32]
Q2	16.92	0.377	[-20.63, 54.47]	1.43	0.918	[-25.77, 28.62]
Q1	-13.47	0.507	[-53.31, 26.37]	-11.43	0.437	[-40.28, 17.42]
Lycopene		REF			REF	
Q3	-19.11	0.296	[-54.93, 16.72]	-1.19	0.927	[-34.80, 24.49]
Q2	-24.02	0.202	[-60.92, 12.88]	-14.75	0.275	[-41.20, 11.70]
Q1	**-46.00**	**0.020**	**[-84.68, -7.32]**	**-28.09**	**0.047**	**[-55.87, -0.31]**
25-OH Vitamin D	**-26.57**	**0.044**	**[-52.44, -0.71]**	-17.89	0.059	[-36.45, 0.68]
Transferrin: Ferritin Index	42.64	0.064	[-2.41, 87.69]	2.01	0.904	[-30.55, 34.56]
Selenium	**-38.72**	**0.004**	**[-64.84, -12.59]**	**-24.01**	**0.012**	**[-42.82, -5.21]**

*Models are defined as follows:

Model C: Adjusted for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin

Model D: Model C + adjustment for screening CD4

Table 7a. Inflammatory biomarkers associated with CD4 recovery at 96 weeks (A & B)*					
	MODEL A			MODEL B	
Biomarker	**Coefficient**	**P-value**	**95% CI**	**Coefficient**	**P-value**	**95% CI**
CRP		REF			REF	
Q2	4.62	0.798	[-30.73, 39.97]	3.87	0.772	[-22.27, 30.02]
Q3	**-37.08**	**0.040**	**[-72.45, -1.71]**	-9.74	0.469	[-36.11, 16.63]
Q4	**-62.98**	**<0.001**	**[-98.24, -27.72]**	-23.57	0.081	[-50.05, 2.91]
LPS	-22.03	0.082	[-46.86, 2.81]	2.02	0.831	[-16.49, 20.53]
sCD14		REF			REF	
Q2	35.70	0.054	[-0.63, 72.02]	26.07	0.054	[-0.41, 52.56]
Q3	27.60	0.138	[-8.85, 64.04]	22.72	0.094	[-3.86, 49.29]
Q4	-2.50	0.890	[-38.10, 33.09]	19.26	0.148	[-6.83, 45.35]
Endocab		REF			REF	
Q2	8.55	0.640	[-27.26, 44.35]	4.19	0.752	[-21.85, 30.24]
Q3	13.30	0.466	[-22.47, 49.07]	14.77	0.266	[-11.24, 40.78]
Q4	-0.61	0.974	[-36.41, 35.20]	-0.39	0.976	[-26.43, 25.65]
Interferon-γ		REF			REF	
Q2	-13.35	0.487	[-50.99, 24.28]	2.49	0.859	[-24.91, 29.89]
Q3	-32.34	0.094	[-70.13, 5.45]	-6.32	0.654	[-33.95, 21.30]
Q4	**-46.31**	**0.016**	**[-84.01, -8.62]**	0.355	0.980	[-27.52, 28.22]
IL-6		REF			REF	
Q2	-33.52	0.080	[-71.09, 4.05]	-2.66	0.850	[-30.25, 24.93]
Q3	**-56.00**	**0.003**	**[-93.44, -18.57]**	-5.94	0.676	[-33.78, 21.90]
Q4	**-39.81**	**0.037**	**[-77.32, -2.31]**	0.23	0.987	[-27.48, 27.94]
IP-10		REF			REF	
Q2	-13.12	0.495	[-50.86, 24.61]	-0.096	0.995	[-27.33, 27.14]
Q3	-27.40	0.157	[-65.32, 10.52]	-11.34	0.417	[-38.72, 16.03]
Q4	-29.16	0.130	[-66.91, 8.60]	11.33	0.420	[-16.23, 38.90]
TNF-α		REF			REF	
Q2	6.74	0.728	[-31.27, 44.75]	18.93	0.175	[-8.45, 46.30]
Q3	8.73	0.653	[-29.35, 46.81]	15.47	0.268	[-11.92, 42.86]
Q4	-7.97	0.681	[-45.97, 30.03]	18.98	0.176	[-8.51, 46.46]

*Models are defined as follows:

Model A: Univariate

Model B: Adjusted for screening CD4

Table 7b. Inflammatory biomarkers associated with CD4 recovery at 96 weeks (C & D)*					
	MODEL C			MODEL D	
Biomarker	**Coefficient**	**P-value 95% CI**		**Coefficient**	**P-value 95% CI**
CRP		REF			REF
Q2	9.26	0.603	[-25.61, 44.13]	3.65	0.775 [-21.39, 28.69]
Q3	-16.74	0.358	[-52.40, 18.83]	-4.59	0.726 [-30.28, 21.09]
Q4	-32.37	0.088	[-69.56, 4.82]	-18.89	0.167 [-45.67, 7.89]
LPS	-7.40	0.563	[-32.50, 17.70]	16.69	0.074 [-1.65, 35.03]
sCD14		REF			REF
Q2	29.11	0.108	[-6.37, 64.59]	**31.18**	**0.016 [5.84, 56.51]**
Q3	30.08	0.102	[-6.02, 66.18]	**30.52**	**0.020 [4.73, 56.32]**
Q4	11.02	0.554	[-25.46, 47.50]	18.70	0.161 [-7.42, 44.83]
Endocab		REF			REF
Q2	-1.20	0.947	[-36.35, 33.95]	0.16	0.990 [-25.01, 25.32]
Q3	11.50	0.520	[-23.56, 46.56]	11.51	0.369 [-13.57, 36.58]
Q4	0.18	0.992	[-35.22, 35.59]	-4.88	0.706 [-30.23, 20.46]
Interferon-γ		REF			REF
Q2	-7.54	0.693	[-45.03, 29.95]	0.37	0.978 [-25.99, 26.73]
Q3	-25.47	0.183	[-63.0, 12.05]	-11.06	0.412 [-37.50, 15.37]
Q4	-33.42	0.079	[-70.75, 3.91]	-0.01	0.999 [-26.48, 26.46]
IL-6		REF			REF
Q2	-29.66	0.119	[-67.0, 7.67]	-10.58	0.432 [-36.98, 15.82]
Q3	**-52.06**	**0.007**	**[-89.84, -14.28]**	-16.95	0.217 [-43.85, 9.95]
Q4	-30.64	0.109	[-68.11, 6.83]	-5.97	0.660 [-32.55, 20.60]
IP-10		REF			REF
Q2	-12.07	0.524	[-49.18, 25.03]	3.75	0.778 [-22.24, 29.73]
Q3	-23.31	0.226	[-61.0, 14.39]	-14.90	0.268 [-41.26, 11.45]
Q4	-27.56	0.166	[-66.57, 11.45]	-12.96	0.353 [-40.30, 14.38]
TNF-α		REF			REF
Q2	10.04	0.6	[-27.50, 47.58]	11.58	0.389 [-14.75, 37.91]
Q3	22.30	0.248	[-15.56, 60.16]	6.94	0.609 [-19.64, 33.51]
Q4	11.14	0.578	[-28.11, 50.39]	5.07	0.718 [-22.46, 32.61]

*Models are defined as follows:

Model C: Adjusted for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin

Model D: Model C + adjustment for screening CD4

Table 8. Association of established nutritional deficiencies and clinical CD4 recovery by 96 weeks*□

	MODEL A			MODEL B		
	Odds Ratio	**P-value**	**95% CI**	**Odds Ratio**	**P-value**	**95% CI**
Vitamin B12	**1.87**	**0.043**	**[1.02, 3.44]**	**1.93**	**0.034**	**[1.05, 3.56]**
Vitamin B6	1.07	0.707	[0.739, 1.56]	1.09	0.653	[0.749, 1.59]
Vitamin A (Retinol)	0.92	0.854	[0.365, 2.30]	0.92	0.863	[0.367, 2.31]
25(OH)-Vitamin D	1.02	0.921	[0.724, 1.43]	1.01	0.933	[0.722, 1.43]
Ferritin	1.42	0.272	[0.759, 2.66]	1.33	0.379	[0.703, 2.52]
Transferrin Receptor-Ferritin Index	1.47	0.161	[0.857, 2.54]	1.42	0.212	[0.820, 2.45]
Selenium	1.40	0.056	[0.991, 1.98]	**1.43**	**0.042**	**[1.01, 2.03]**

	MODEL C			MODEL D		
	Odds Ratio	**P-value**	**95% CI**	**Odds Ratio**	**P-value**	**95% CI**
Vitamin B12	**1.97**	**0.034**	**[1.05, 3.69]**	**2.00**	**0.031**	**[1.07, 3.73]**
Vitamin B6	0.93	0.706	[0.627, 1.37]	0.94	0.741	[0.632, 1.38]
Vitamin A (Retinol)	0.74	0.538	[0.279, 1.95]	0.72	0.512	[0.274, 1.91]
25(OH)-Vitamin D	1.02	0.915	[0.707, 1.47]	1.02	0.905	[0.709, 1.47]
Ferritin	1.24	0.546	[0.619, 2.47]	1.19	0.626	[0.591, 2.40]
Transferrin Receptor-Ferritin Index	1.33	0.346	[0.733, 2.42]	1.30	0.394	[0.712, 2.37]
Selenium	**1.52**	**0.028**	**[1.05, 2.21]**	**1.54**	**0.024**	**[1.06, 2.24]**

*Models are defined as follows:

Model A: Univariate

Model B: Adjusted for screening CD4

Model C: Adjusted for race, sex, age, BMI category, treatment regimen, presence of TB at baseline, viral load at baseline, and baseline hemoglobin

Model D: Model C + adjustment for screening CD4

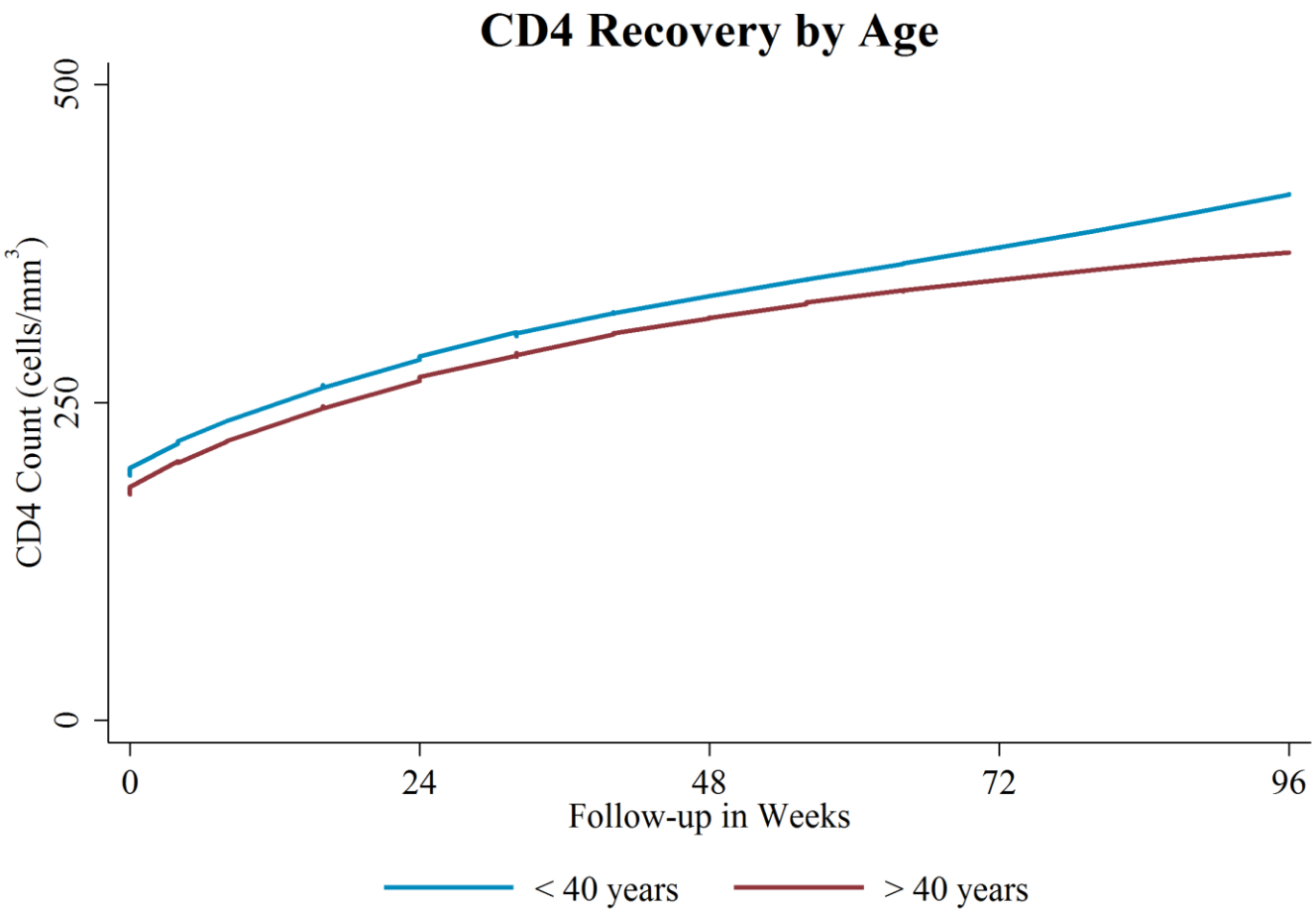
APPENDIX A.

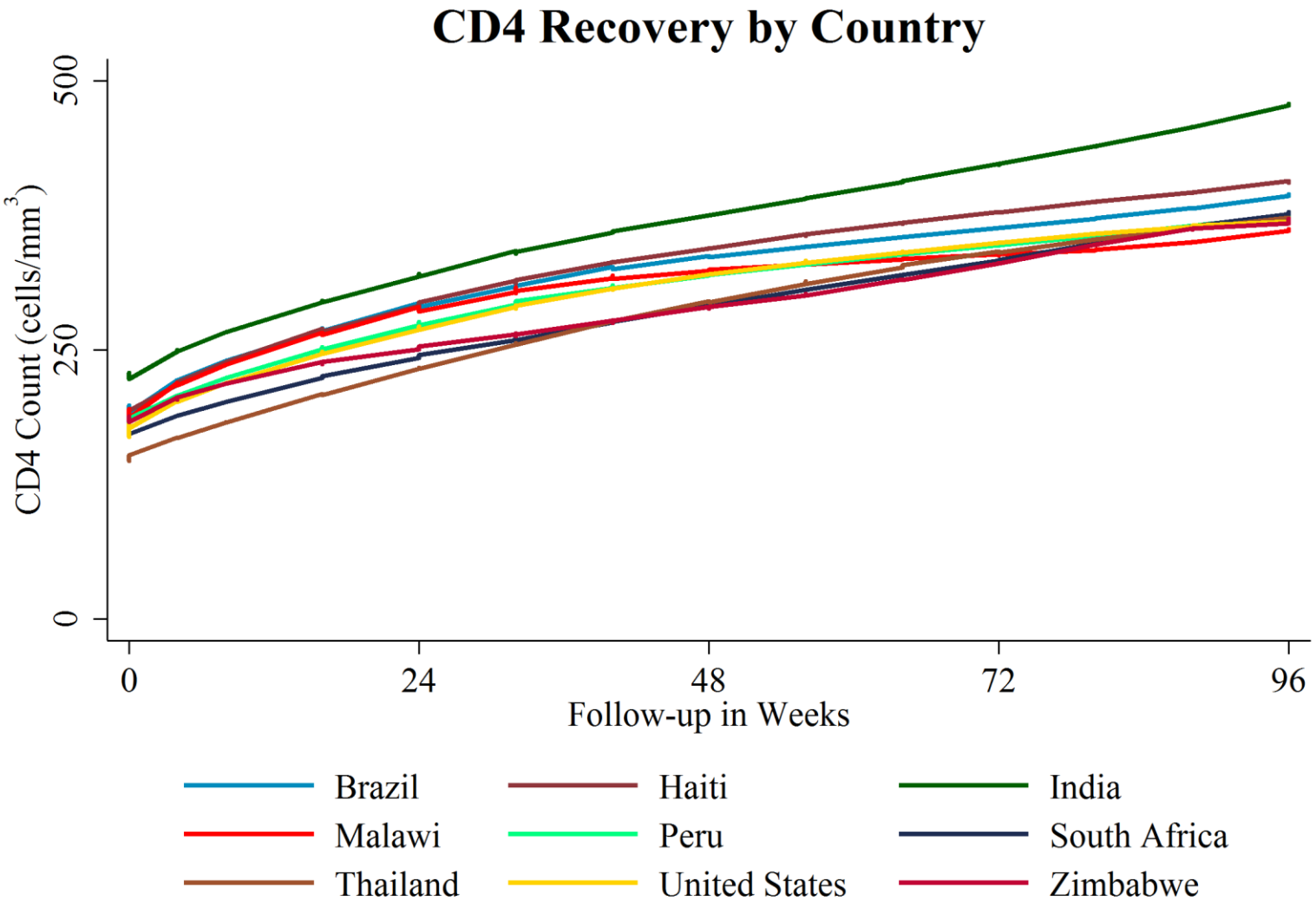
Univariate analysis of CD4 recovery by regression model covariates			
	Coefficient	**P-value**	**95% CI**
Baseline CD4 count (cells/mm^3)	**1.00**	**<0.001**	**[0.96, 1.04]**
Race/Ethnicity			
White		REF	
Black	**-16.11**	**0.085**	**[-34.41, 2.20]**
Hispanic	**-9.11**	**0.371**	**[-29.08, 10.85]**
Asian	**25.48**	**0.008**	**[6.75, 44.21]**
Sex	**41.66**	**<0.001**	**[33.68, 49.65]**
Age (<40)	**-20.40**	**<0.001**	**[-29.25, -11.56]**
BMI Category			
Underweight		REF	
Normal	**-45.52**	**<0.001**	**[-58.14, -32.89]**
Overweight	**-18.20**	**0.015**	**[-32.80, -3.60]**
Treatment			
ddI+FTC+ATV		REF	
TDF/FTC+EFV	**-27.76**	**<0.001**	**[-37.88, -17.64]**
ZDV/3TC+EFV	**-20.10**	**<0.001**	**[-29.77, -10.42]**
TB at baseline	1.39	0.774	[-8.12, 10.90]
Viral load (copies/mL)	**1.98×10^{-5}**	**0.031**	**[1.84E-6, 3.77E-5]**
Baseline hemoglobin (cells/mL)	**6.42**	**<0.001**	**[4.34, 8.51]**

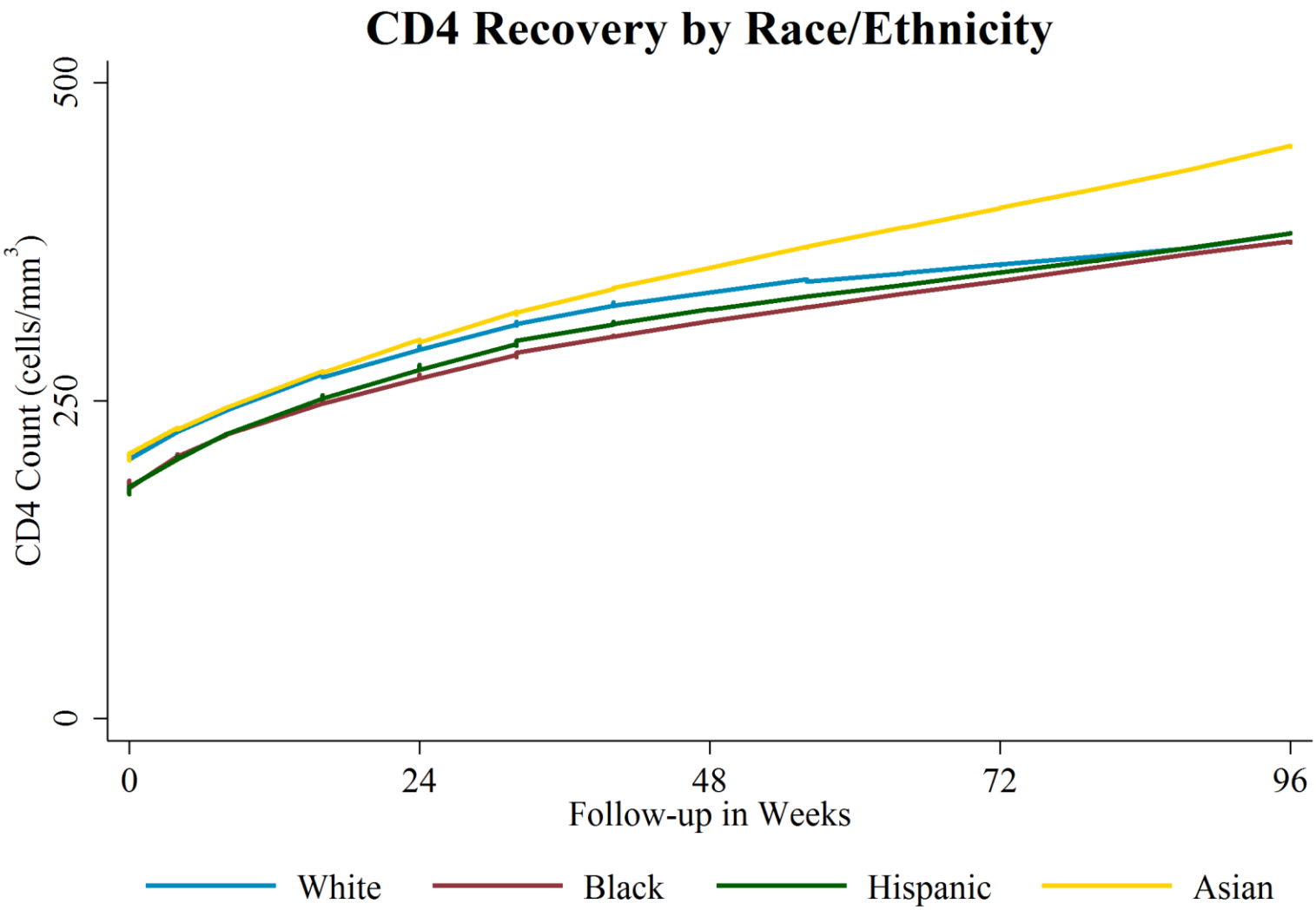
APPENDIX B.

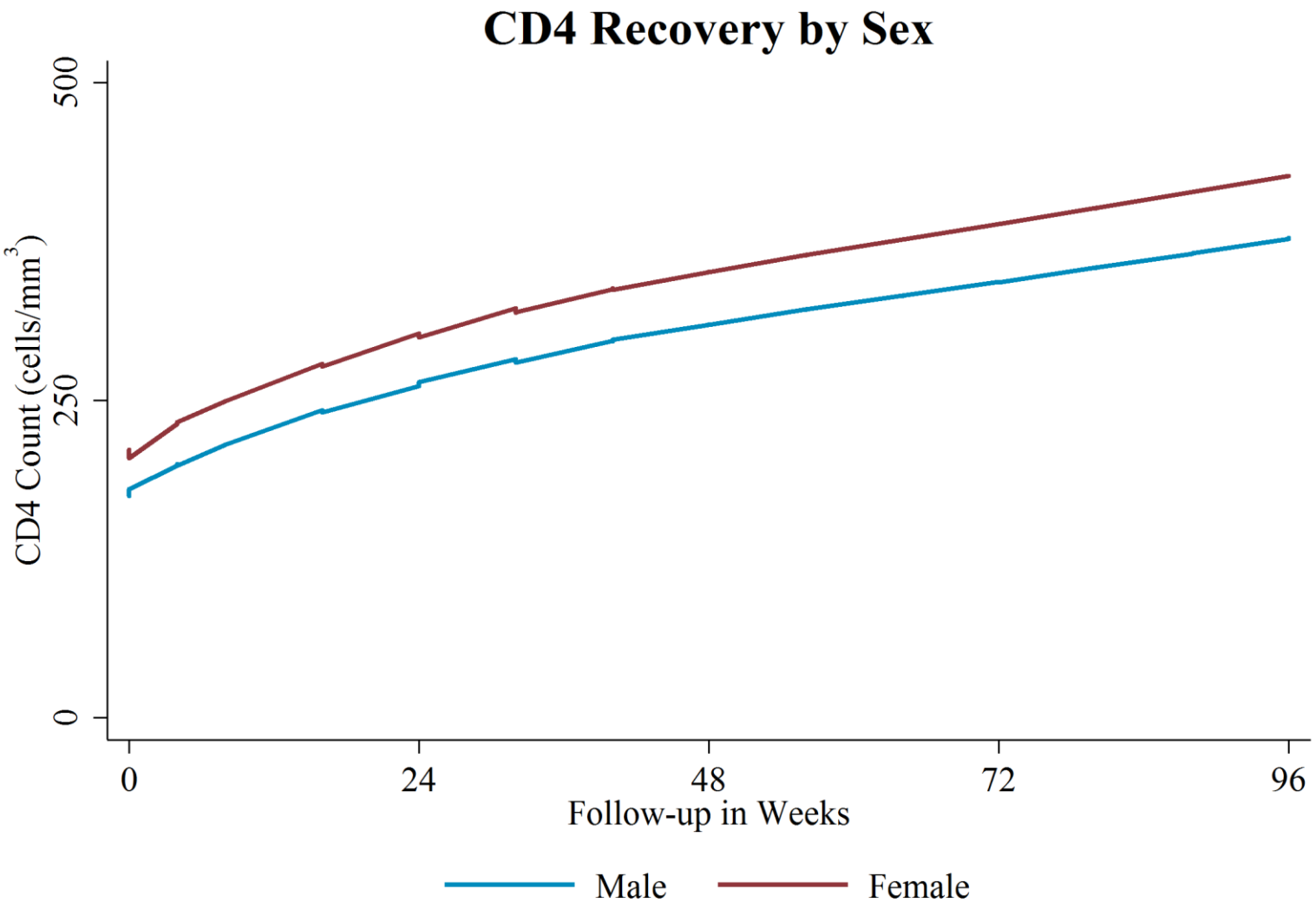
Biomarker	Quartile 1	Quartile 2	Quartile 3	Quartile 4
α-tocopherol (µmol/L)	5.84 - 18.99	19.04 - 22.55	22.57 - 27.05	27.13 - 62.89
γ-tocopherol (µmol/L)	0.72 - 1.65	1.68 - 2.46	2.47 - 3.55	3.56 - 15.04
α-carotene (µmol/L)	0 - 0.02	0.03 - 0.04	0.05 - 0.10	0.11 - 1.82
β-carotene (µmol/L)	0.02 - 0.11	0.12 - 0.21	0.22 - 0.38	0.39 - 3.42
β-cryptoxanthin (µmol/L)	0.01 - 0.03	0.04 - 0.06	0.07 - 0.11	0.12 - 0.96
Lutein (µmol/L)	0.02 - 0.10	0.11 - 0.16	0.17 - 0.25	0.26 - 1.33
Zeaxanthin (µmol/L)	0.01 - 0.02	0.03 - 0.03	0.04 - 0.05	0.06 - 0.26
Lycopene (µmol/L)	0.02 - 0.13	0.14 - 0.28	0.29 - 0.45	0.46 - 3.02
CRP (mg/L)	0.13 - 1.53	1.55 - 3.98	4.19 - 12.95	13.07 - 423.91
sCD14 (ng/mL)	1.06 - 440.1	442.65 - 2000	2100 - 2700	2800 - 5200
Endocab IgM (MMU/ml)	3.43 - 28.95	29 - 45.46	45.52 - 67.7	68.64 - 582.53
Interferon-γ (pg/mL)	0 - 5.69	5.71 - 16.78	16.99 - 45.4	47.97 - 439.93
IL-6 (pg/mL)	0 - 9.54	9.8 - 25.13	25.41 - 49.78	49.98 - 650.63
IP-10 (pg/mL)	53.6 - 616.43	624.9 - 1470.1	1486.5 - 3091.9	3092.4 - 10231.3
TNF-α (pg/mL)	0 - 13.55	13.6 - 19.59	19.73 - 28.38	28.41 - 867.94

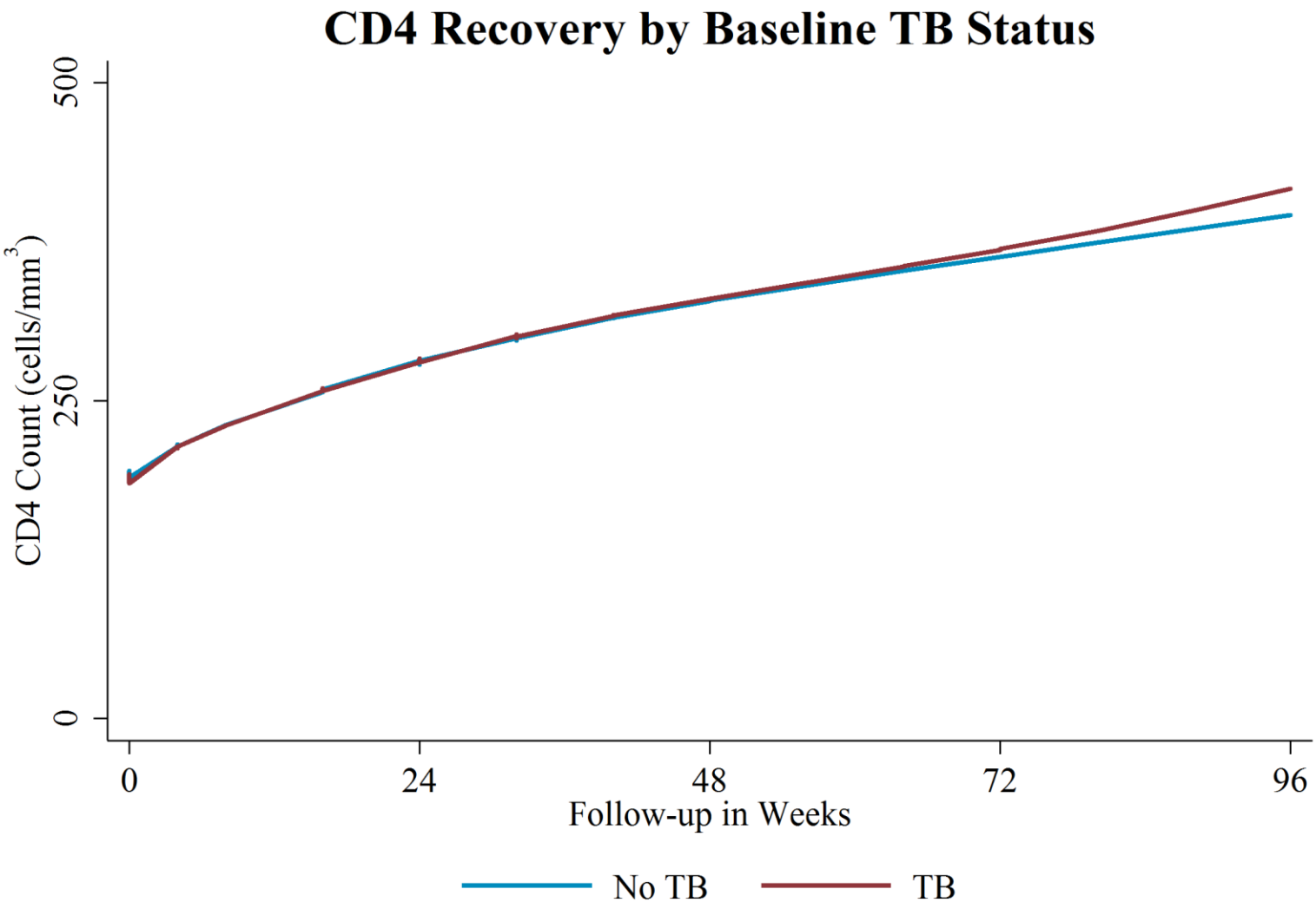
APPENDIX C

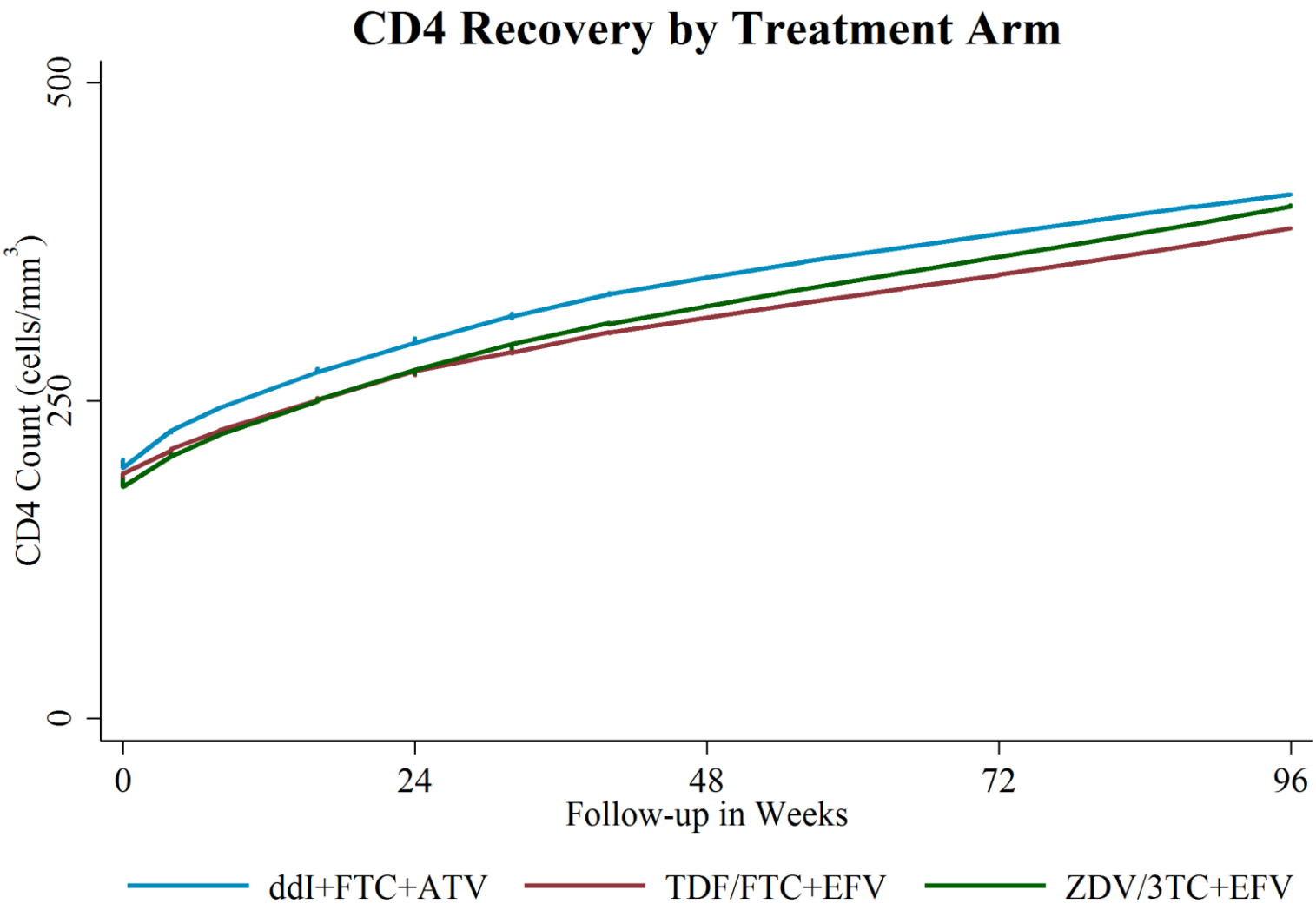

CD4 Recovery by Age
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
< 40 years
> 40 years


CD4 Recovery by Country
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Brazil
Haiti
India
Malawi
Peru
South Africa
Thailand
United States
Zimbabwe

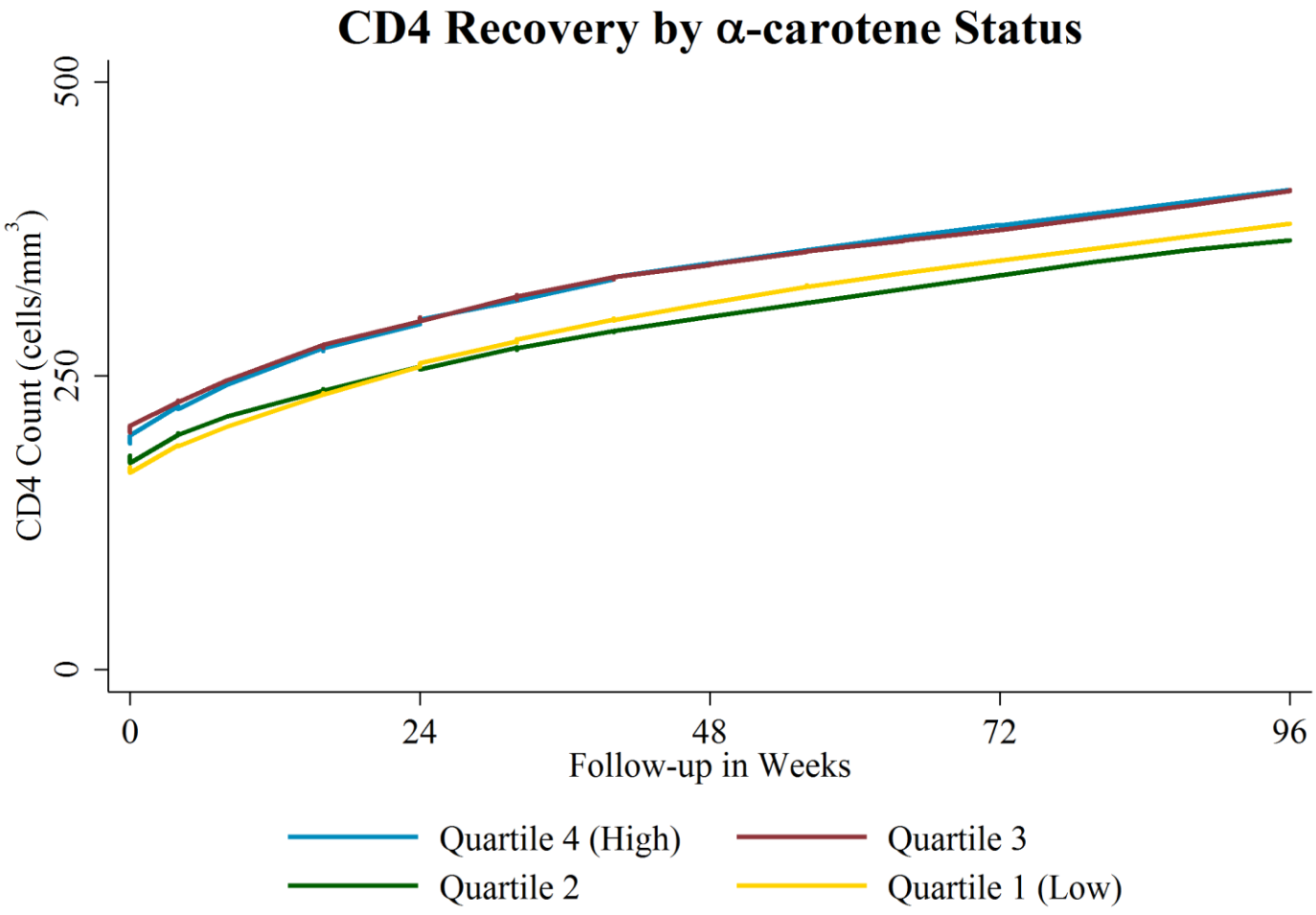

CD4 Recovery by Race/Ethnicity
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
White
Black
Hispanic
Asian

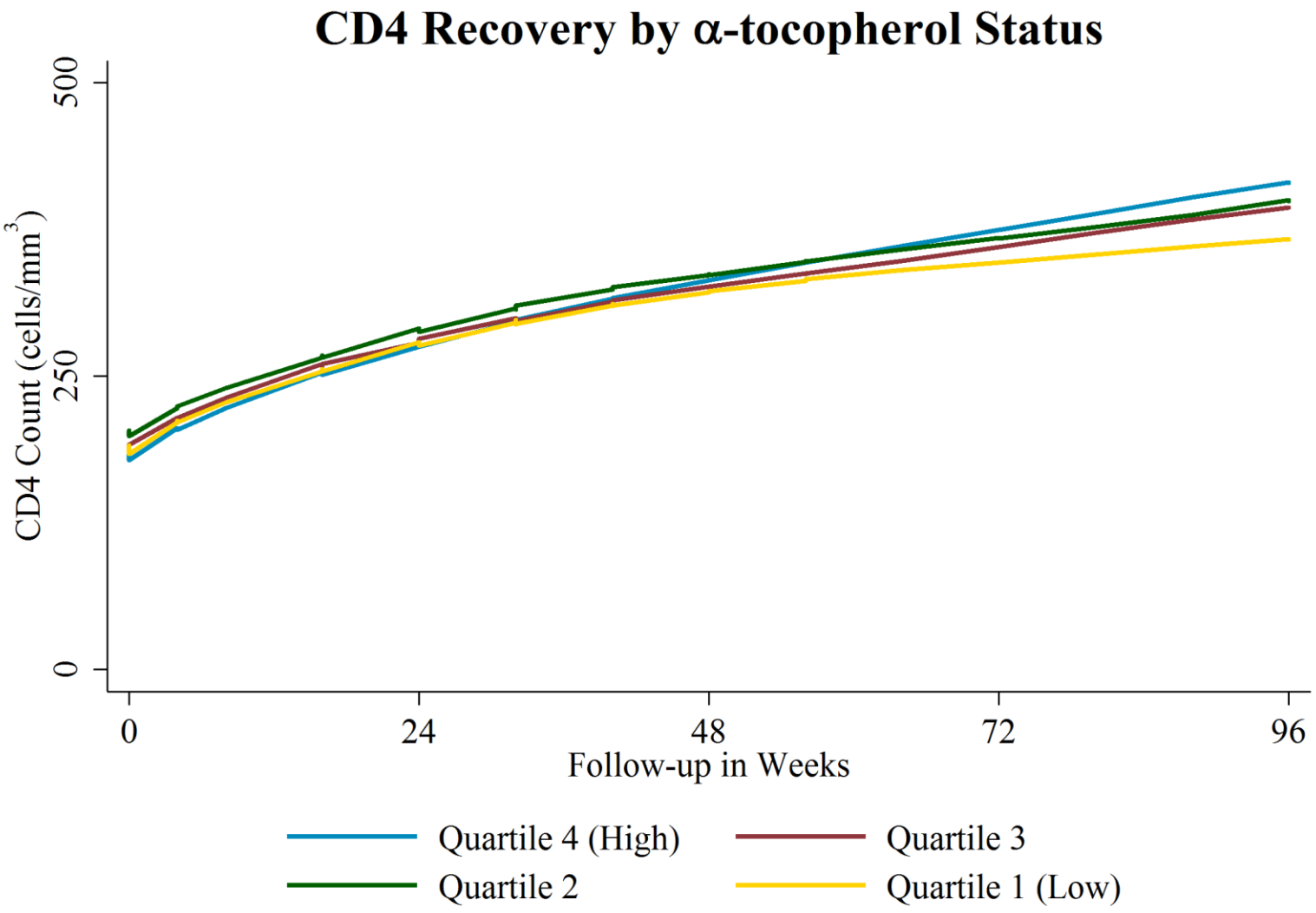

CD4 Recovery by Sex
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Male
Female

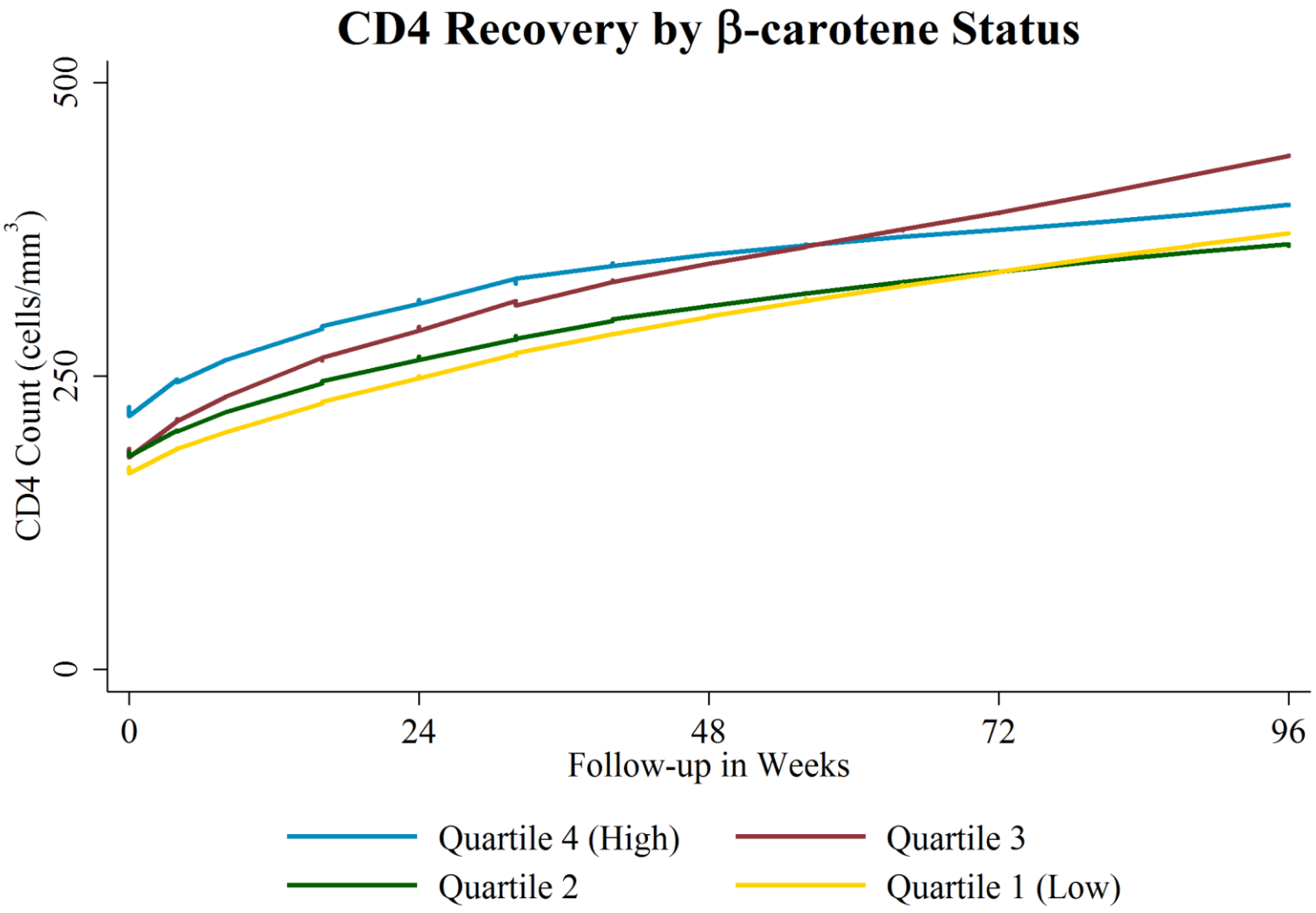

CD4 Recovery by Baseline TB Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
No TB
TB

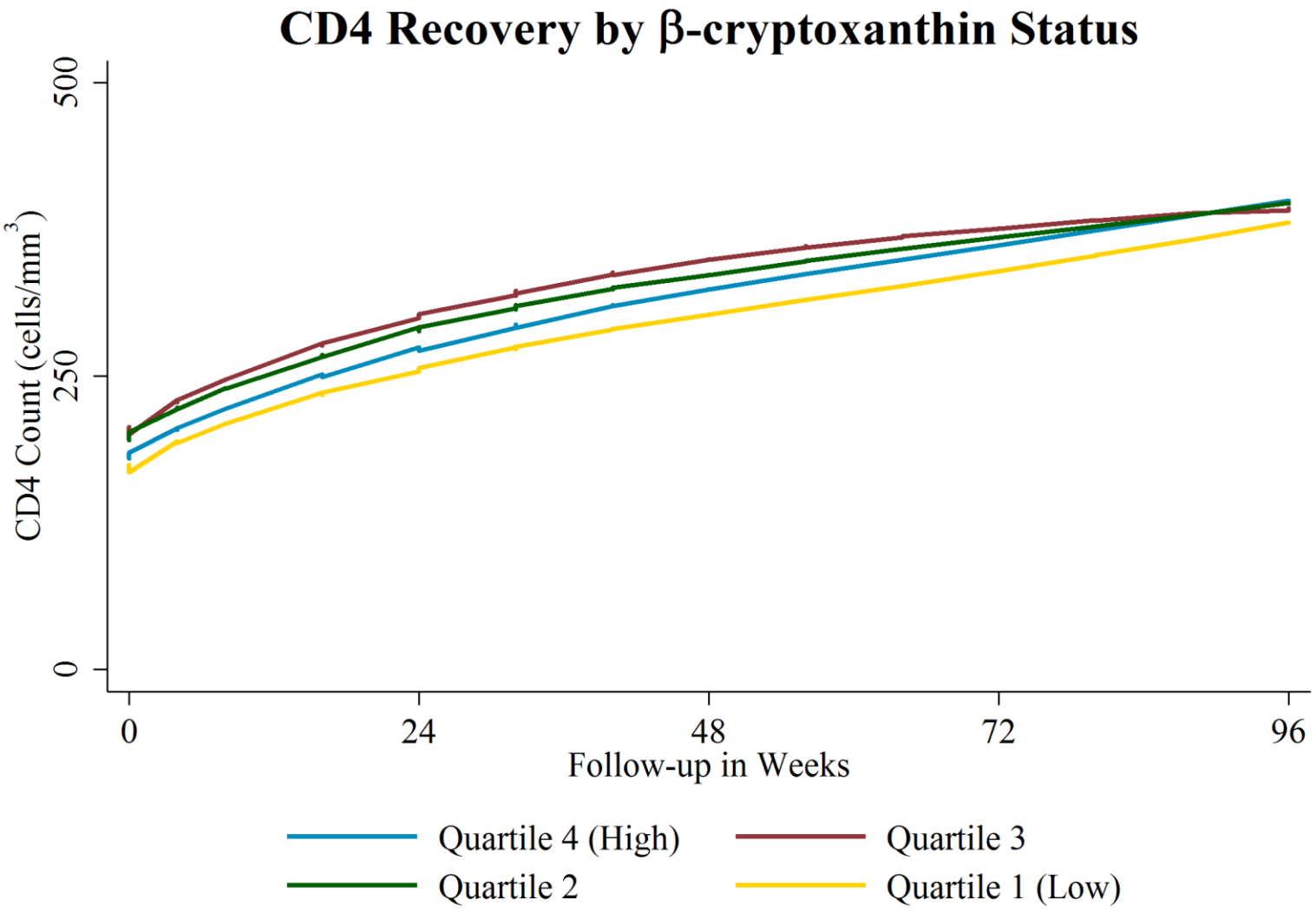

CD4 Recovery by Treatment Arm
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
ddI+FTC+ATV
TDF/FTC+EFV
ZDV/3TC+EFV

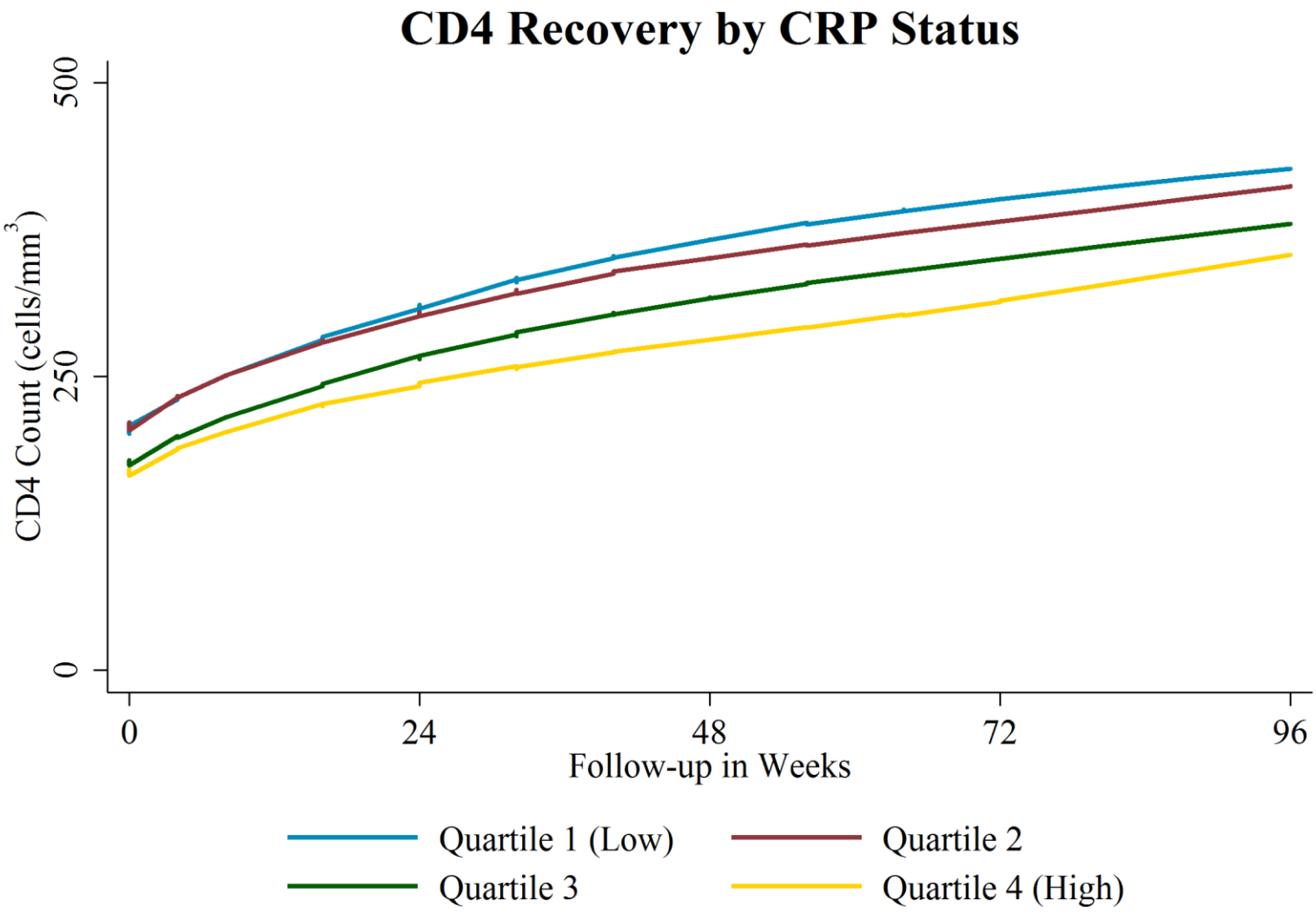
APPENDIX D

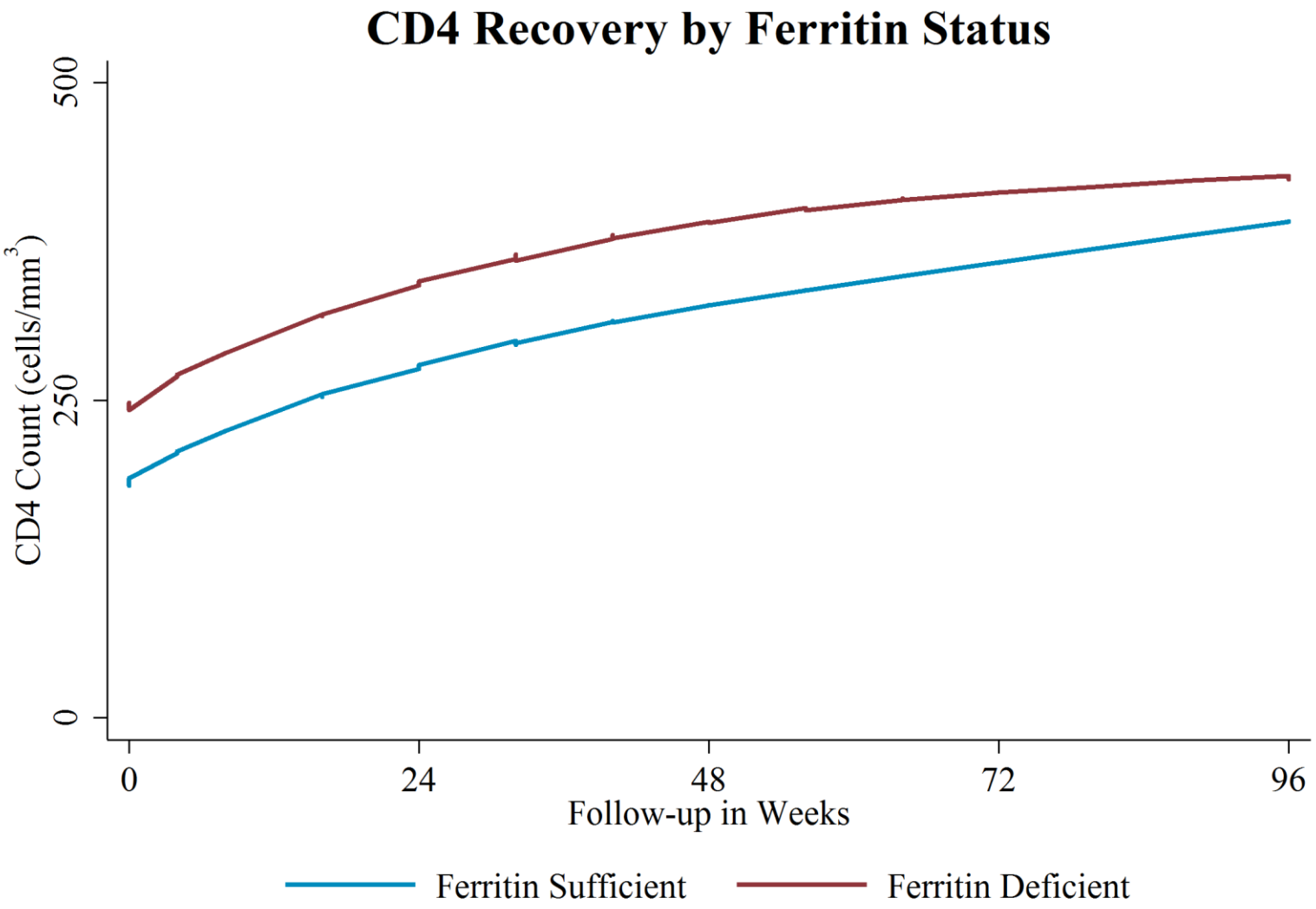

CD4 Recovery by α-carotene Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

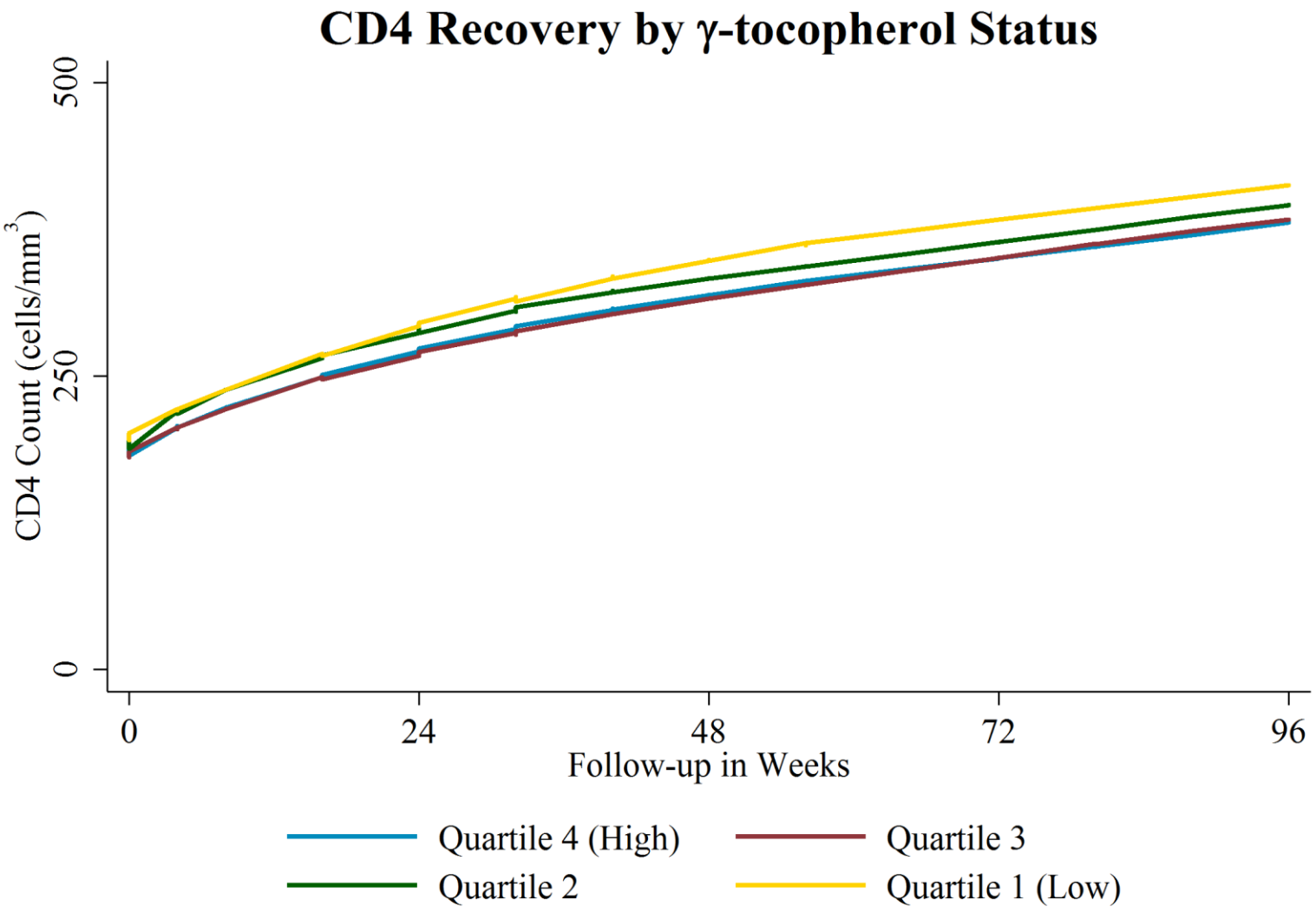

CD4 Recovery by α-tocopherol Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

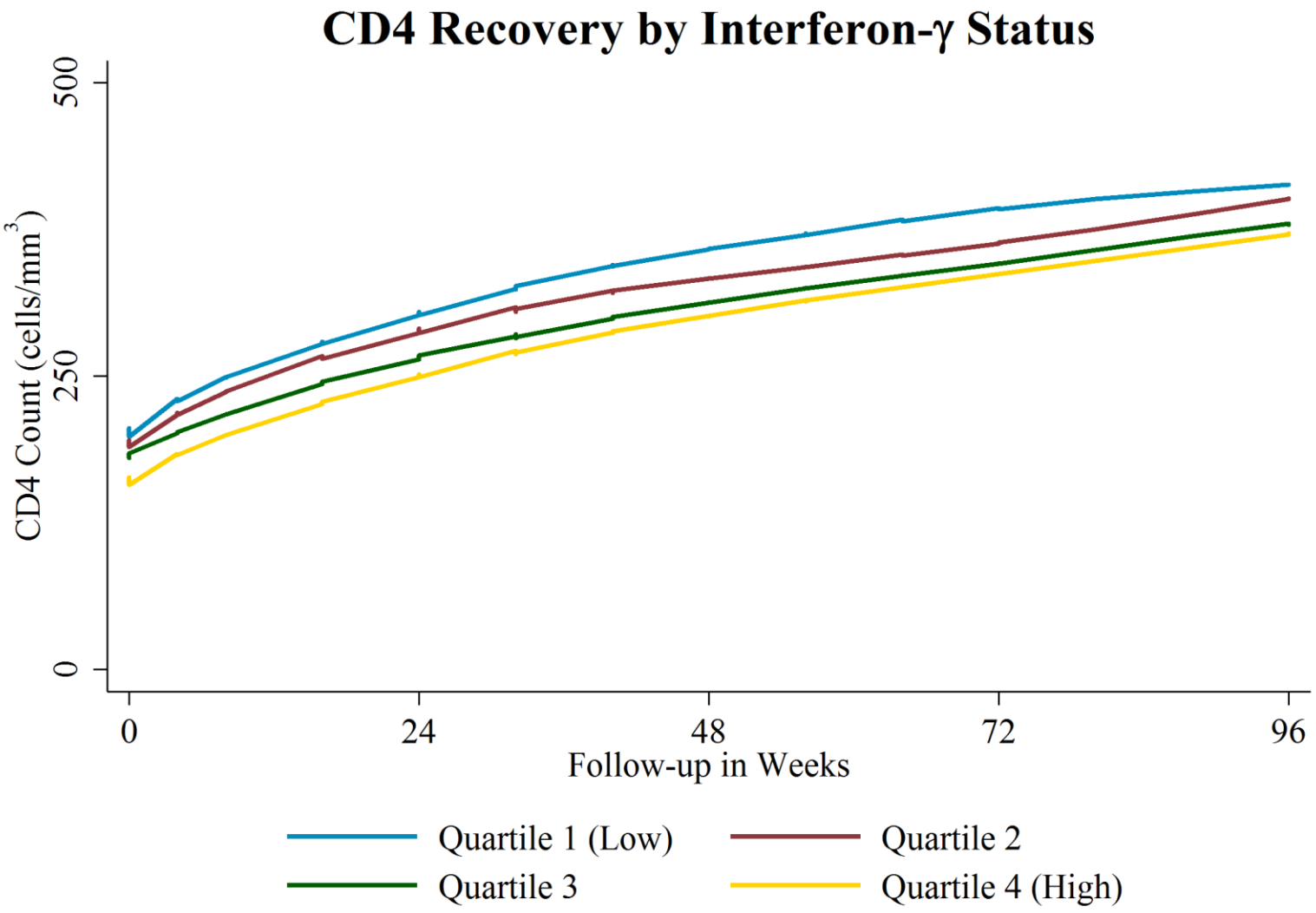

CD4 Recovery by β-carotene Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

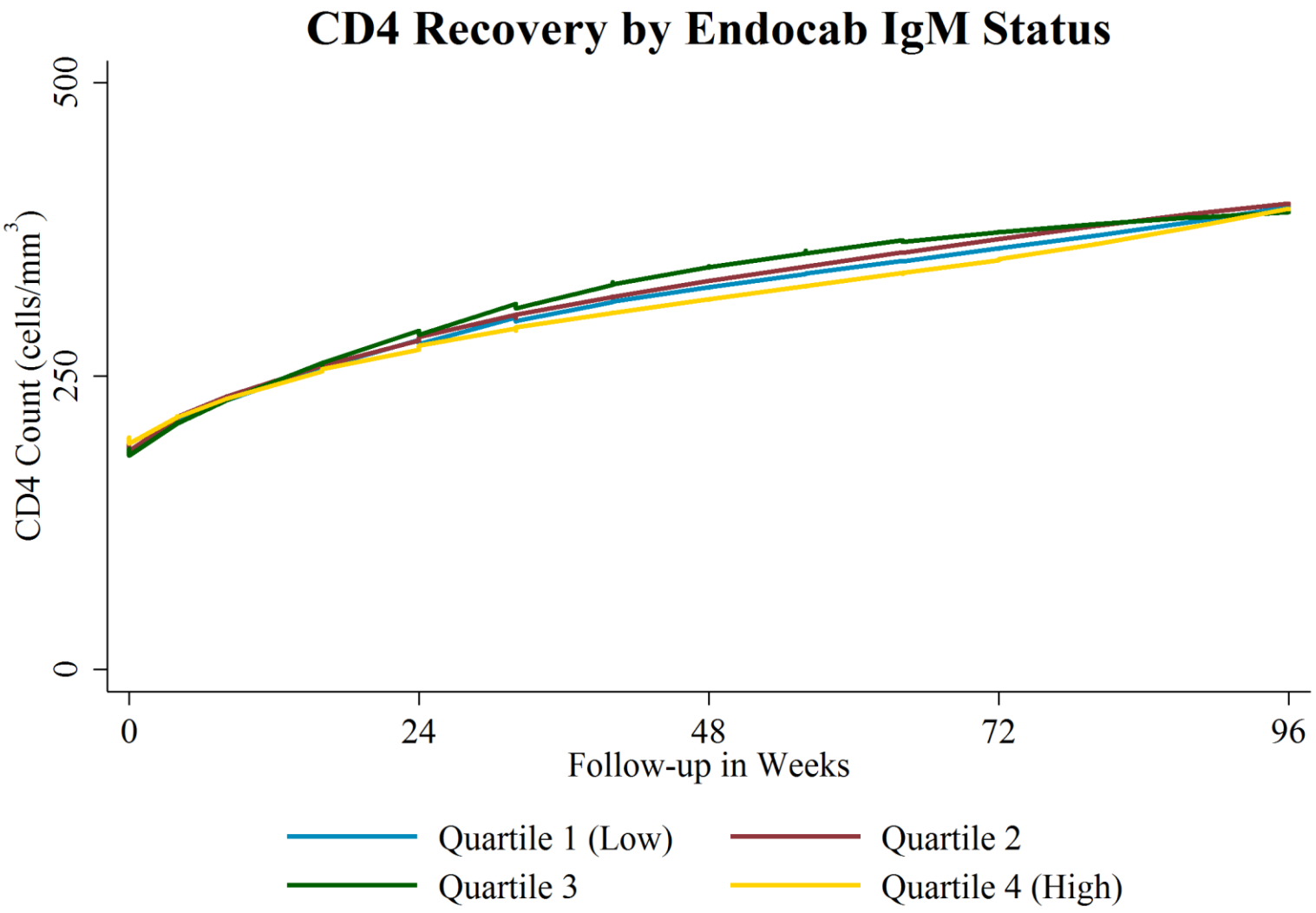

CD4 Recovery by β-cryptoxanthin Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

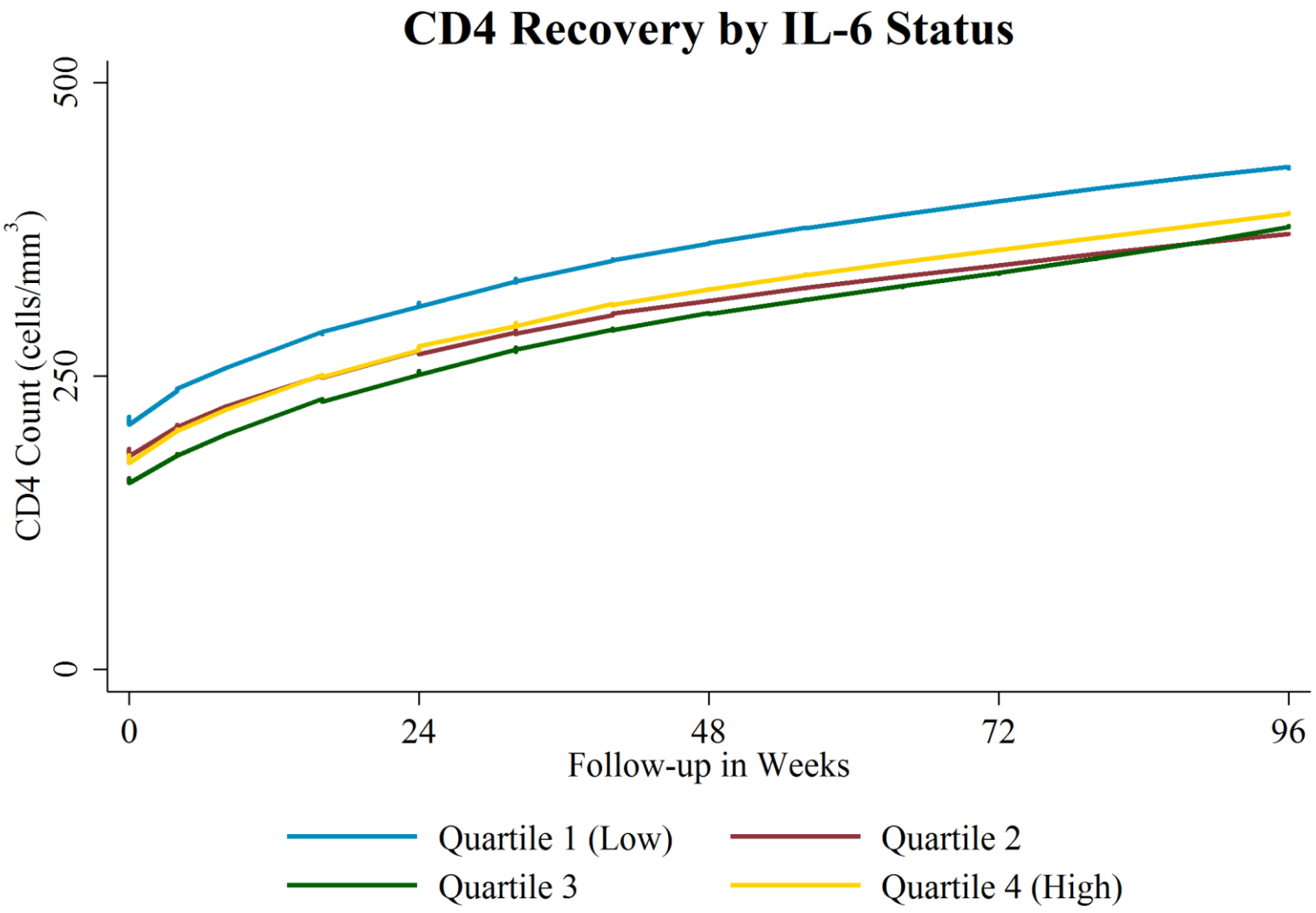

CD4 Recovery by CRP Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

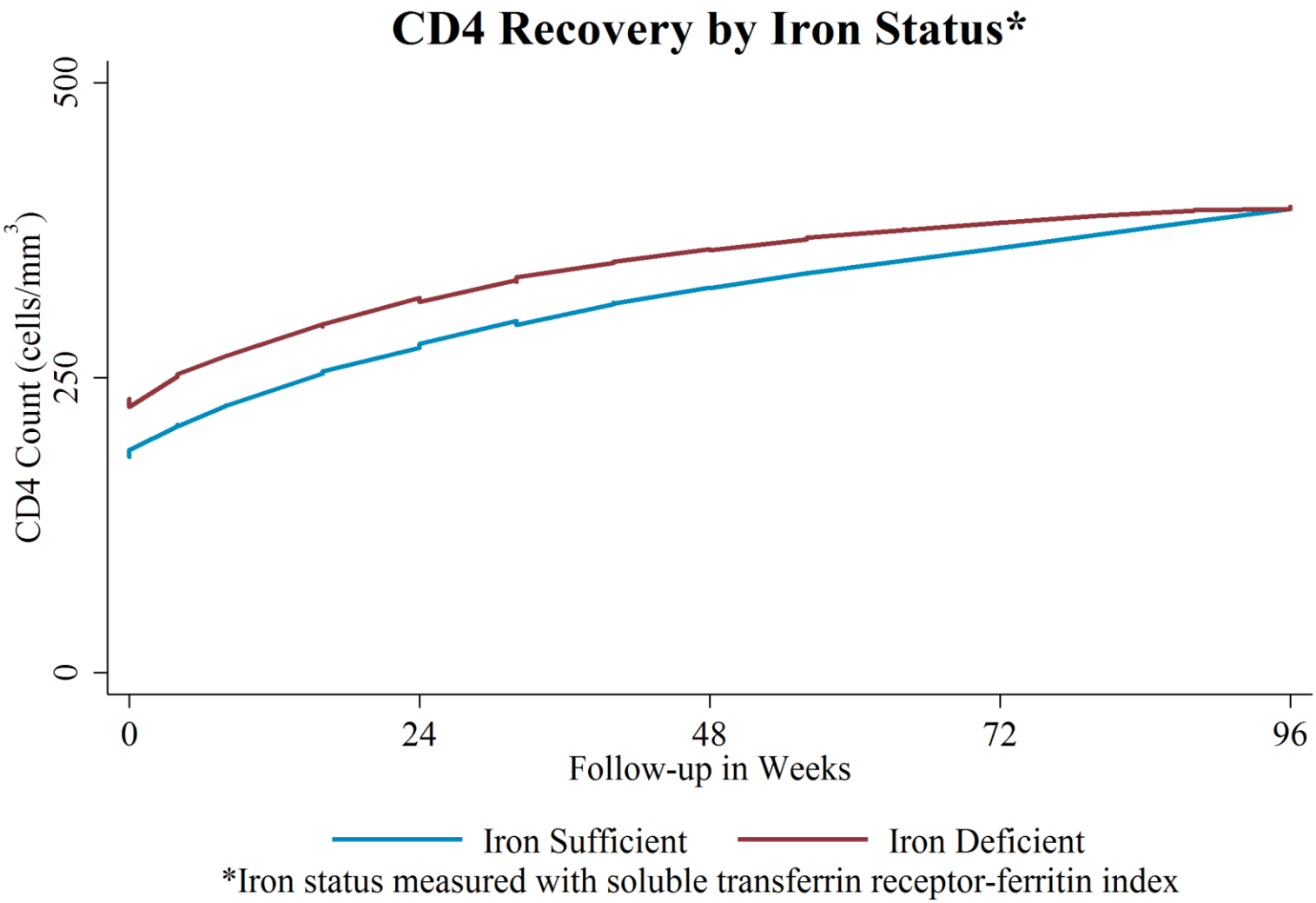

CD4 Recovery by Ferritin Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Ferritin Sufficient
Ferritin Deficient

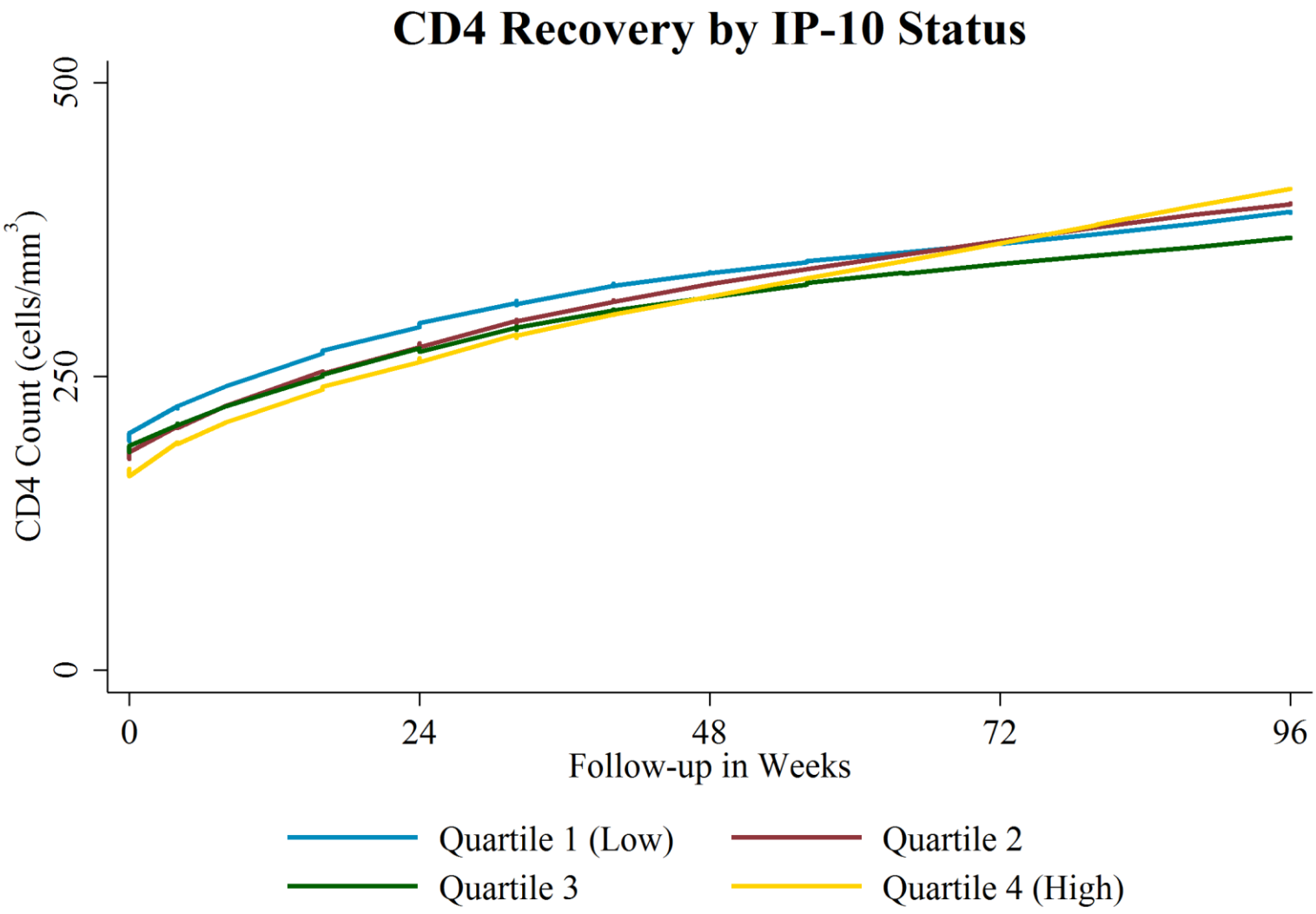

CD4 Recovery by γ-tocopherol Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

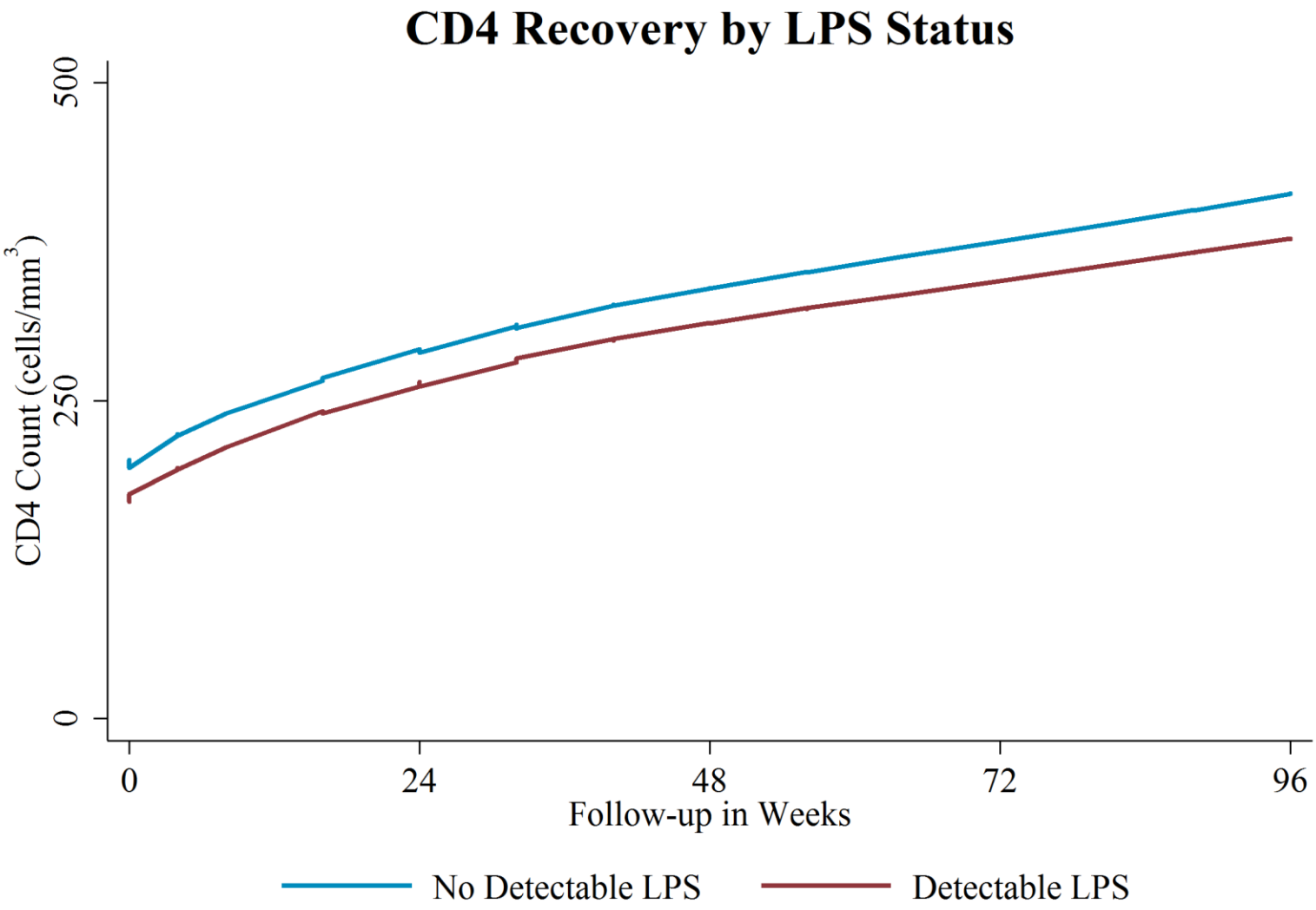

CD4 Recovery by Interferon-γ Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

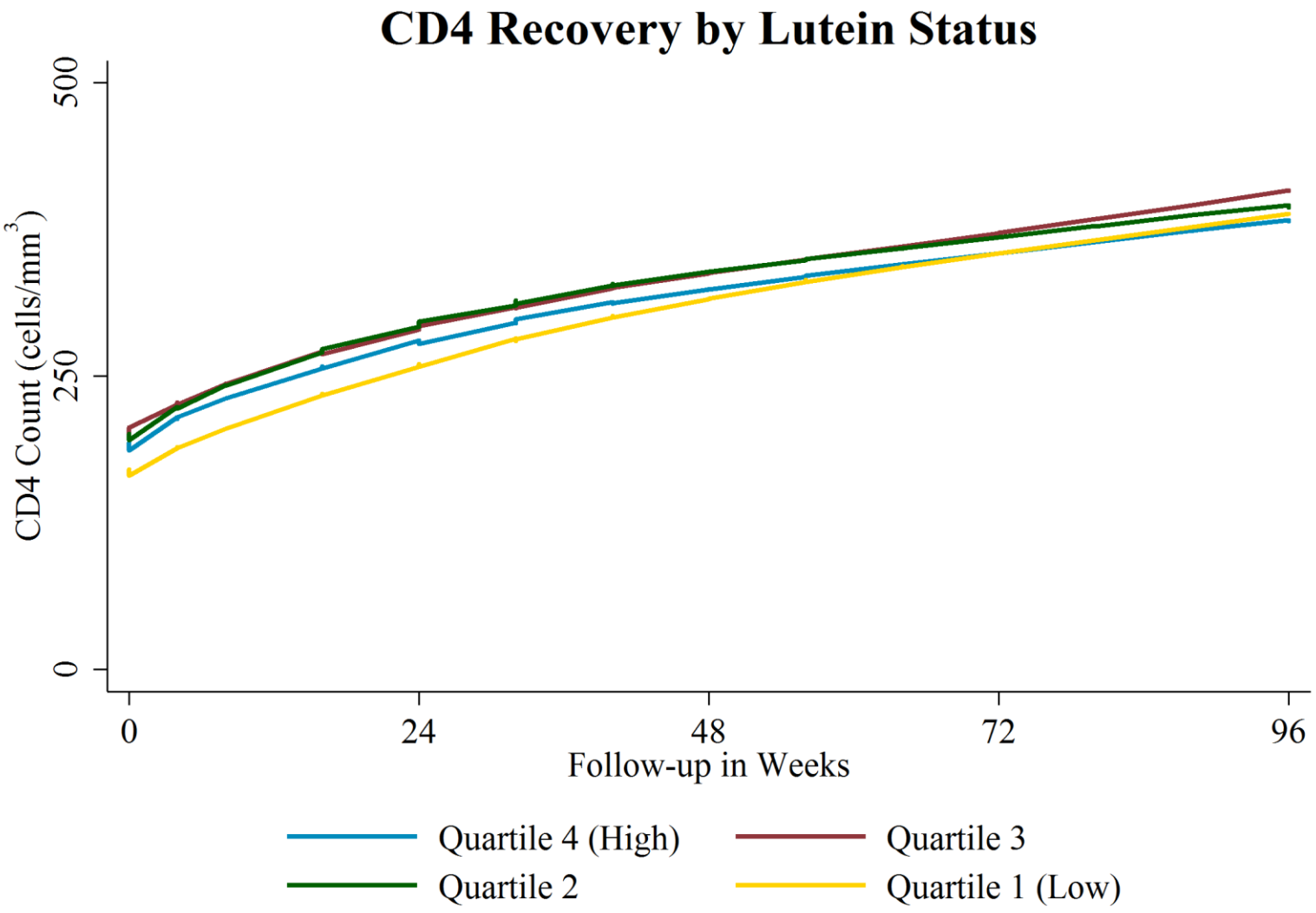

CD4 Recovery by Endocab IgM Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

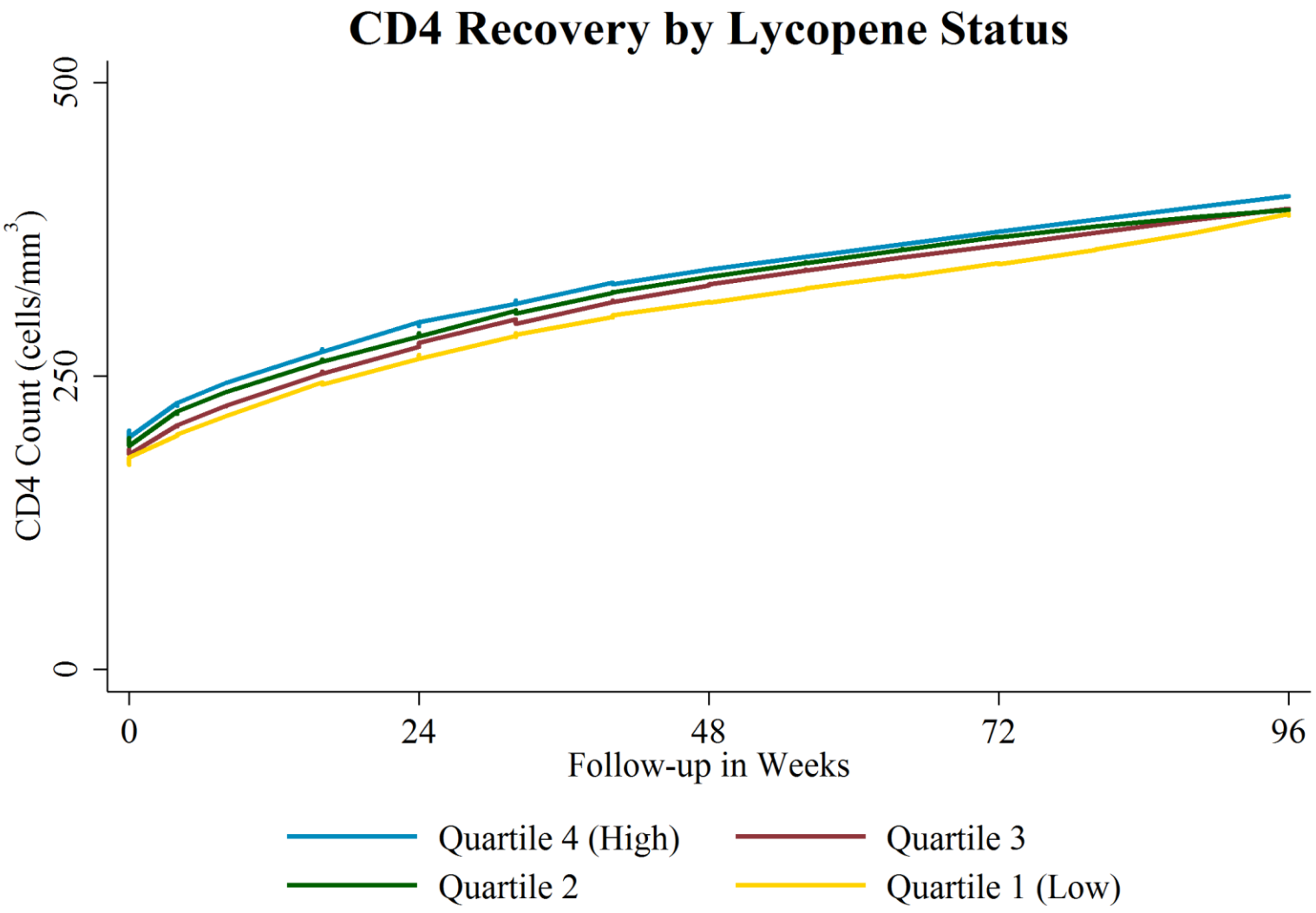

CD4 Recovery by IL-6 Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

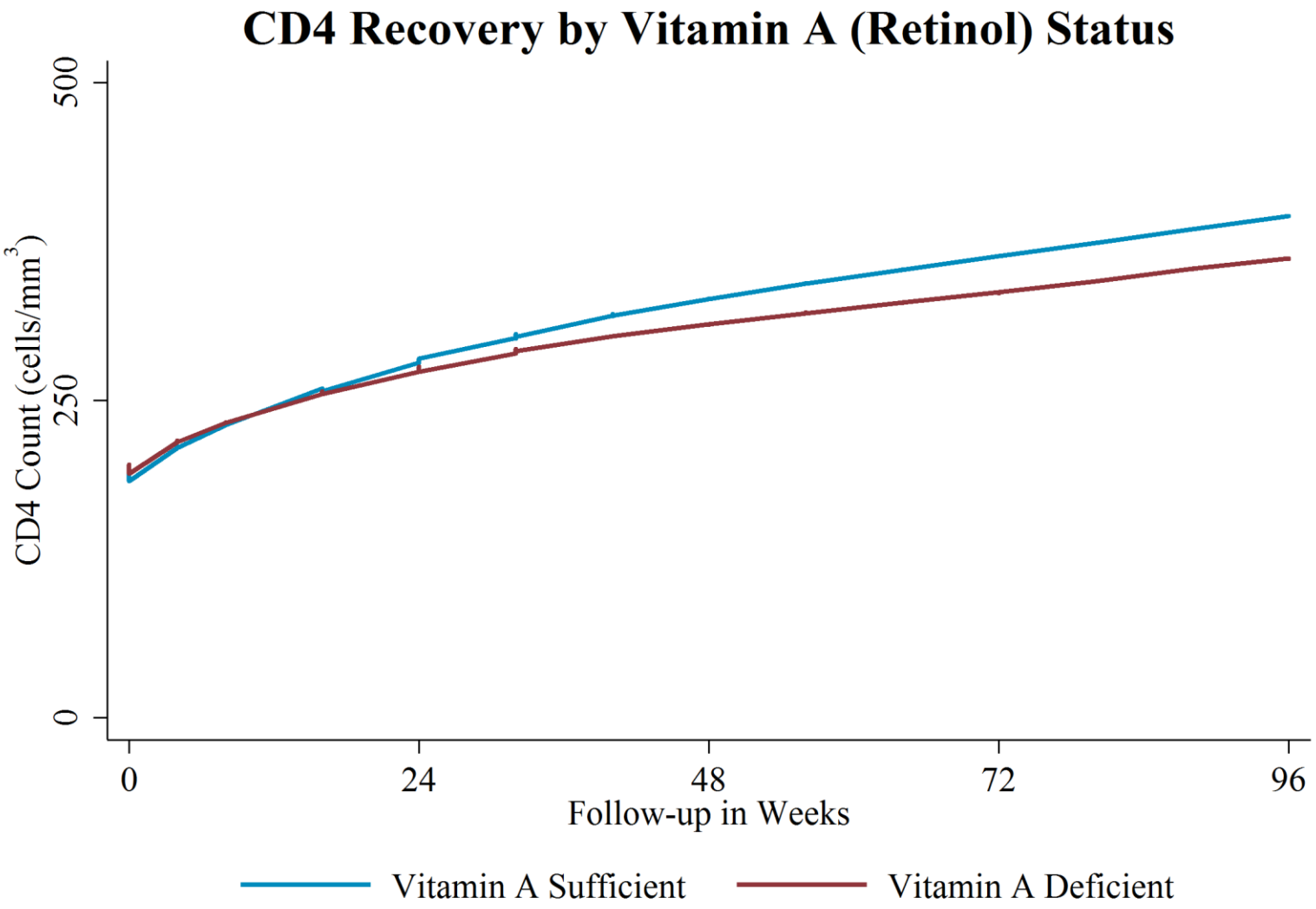

CD4 Recovery by Iron Status*
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Iron Sufficient
Iron Deficient
*Iron status measured with soluble transferrin receptor-ferritin index

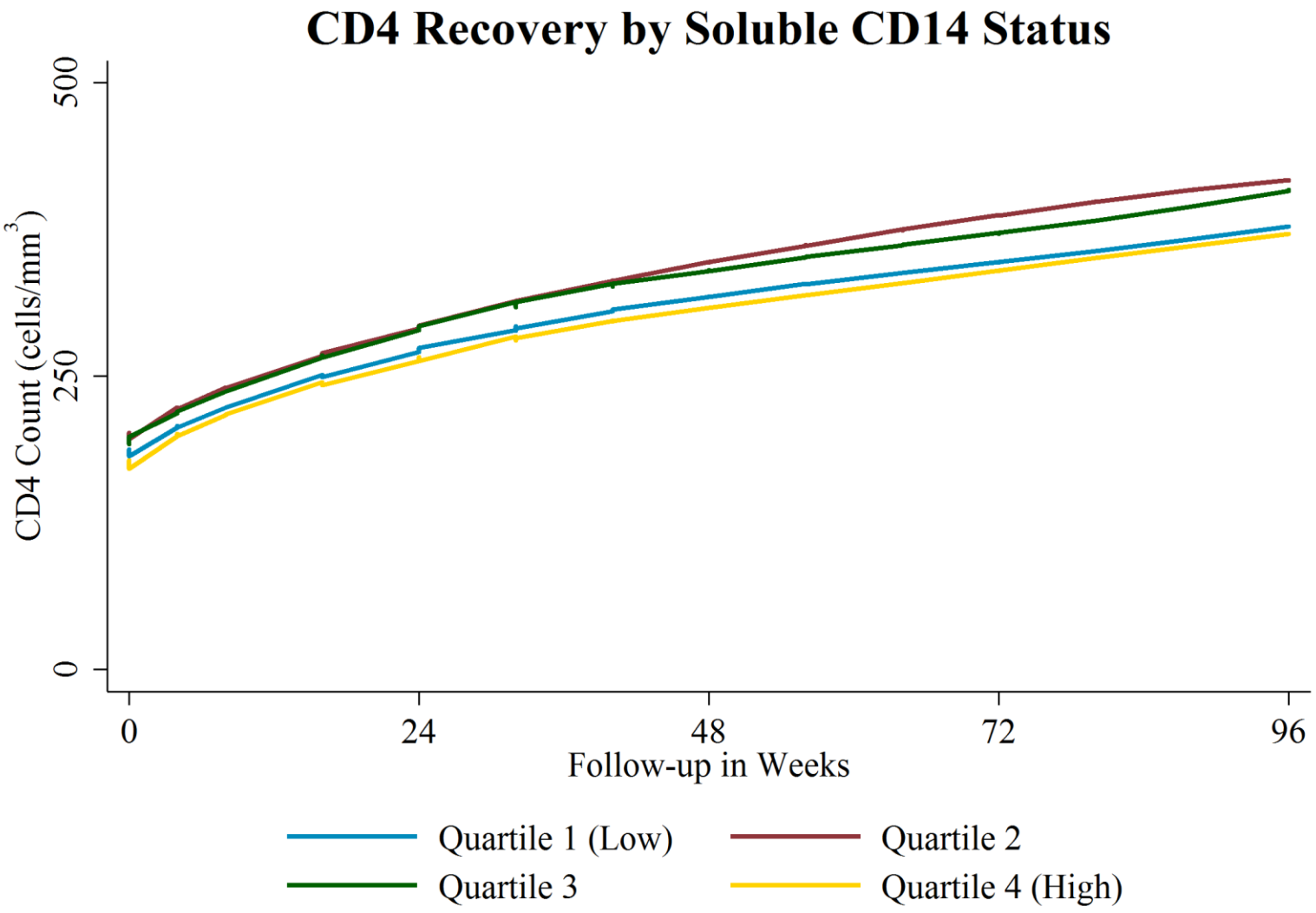

CD4 Recovery by IP-10 Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

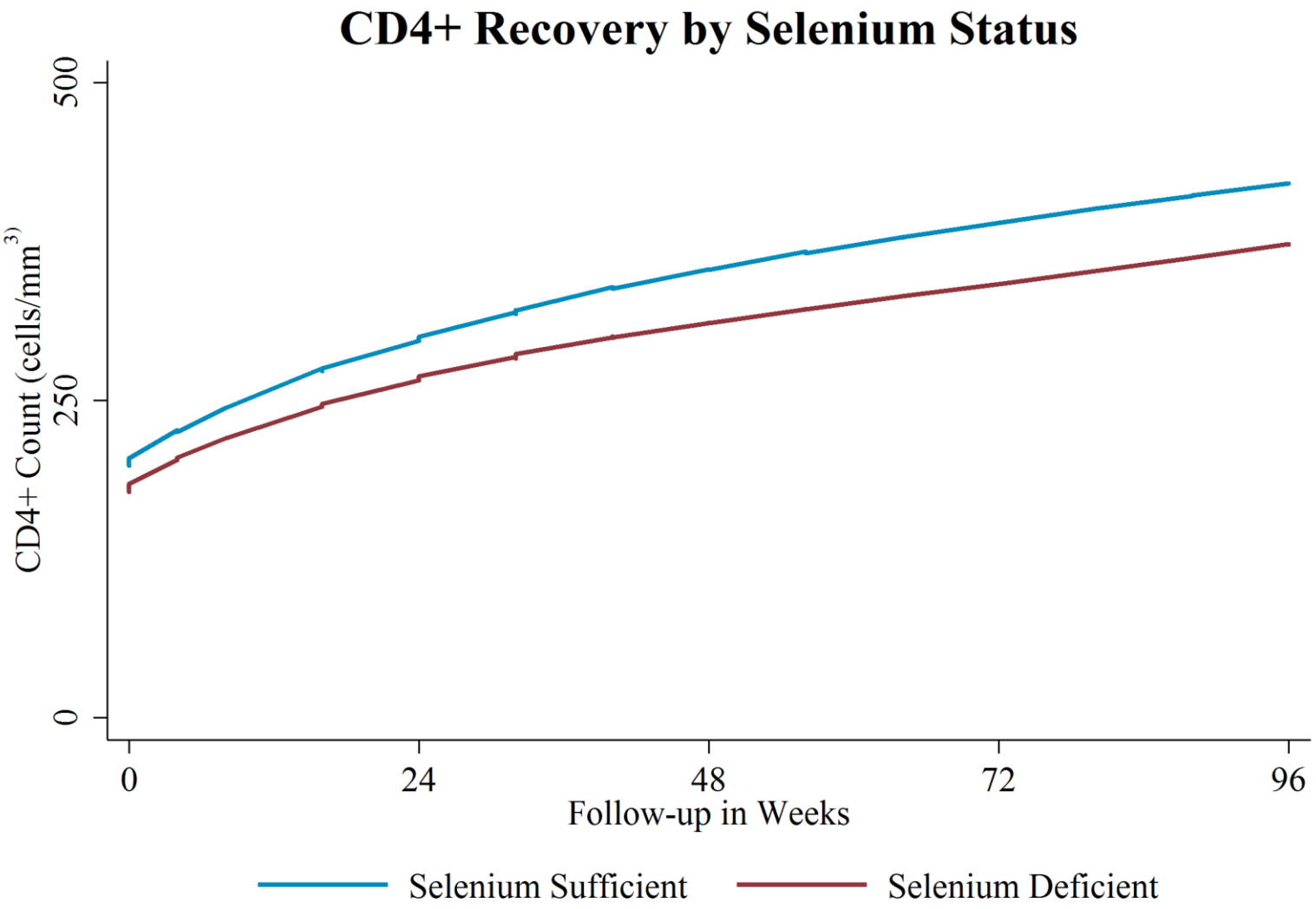

CD4 Recovery by LPS Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
No Detectable LPS
Detectable LPS

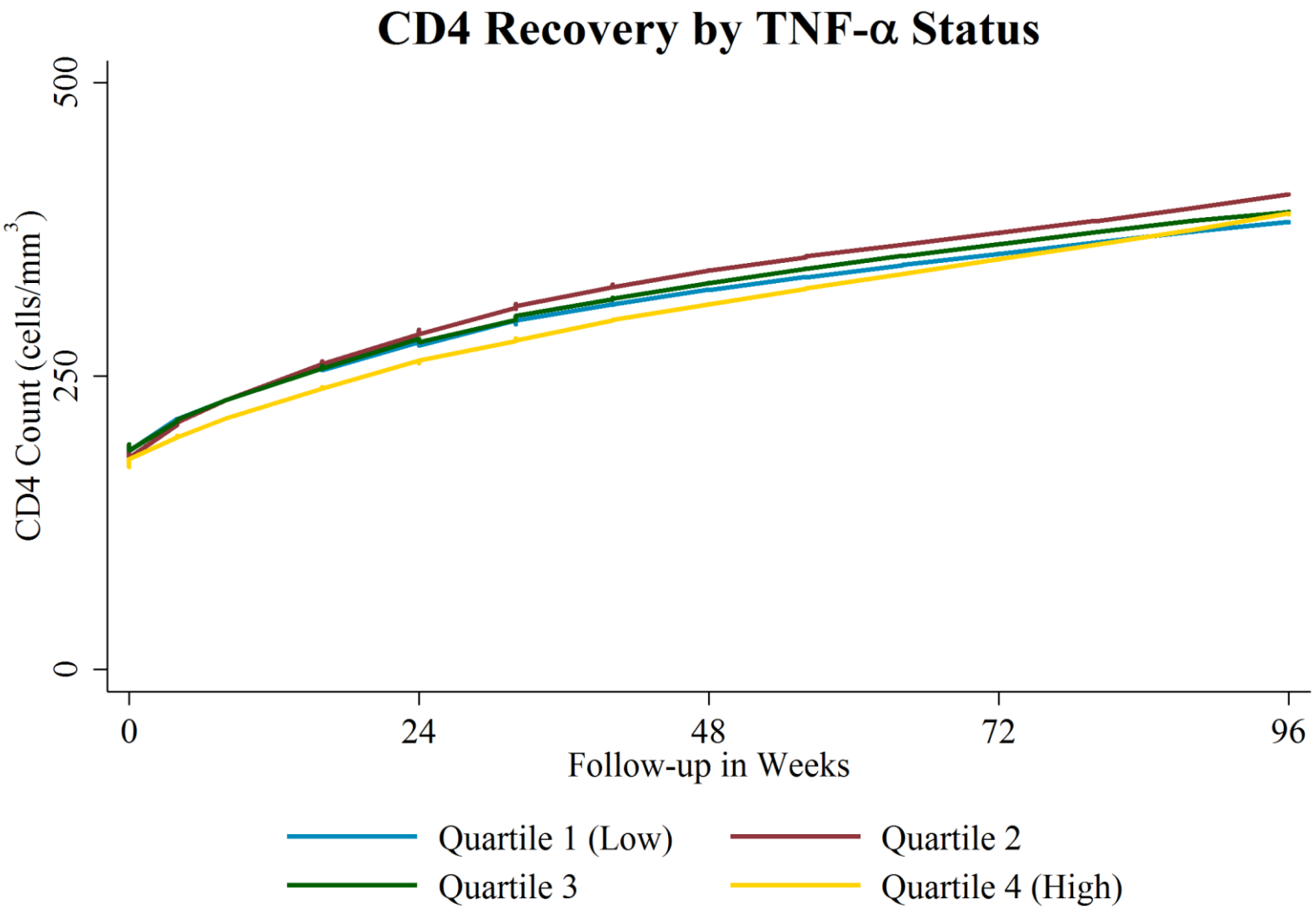

CD4 Recovery by Lutein Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

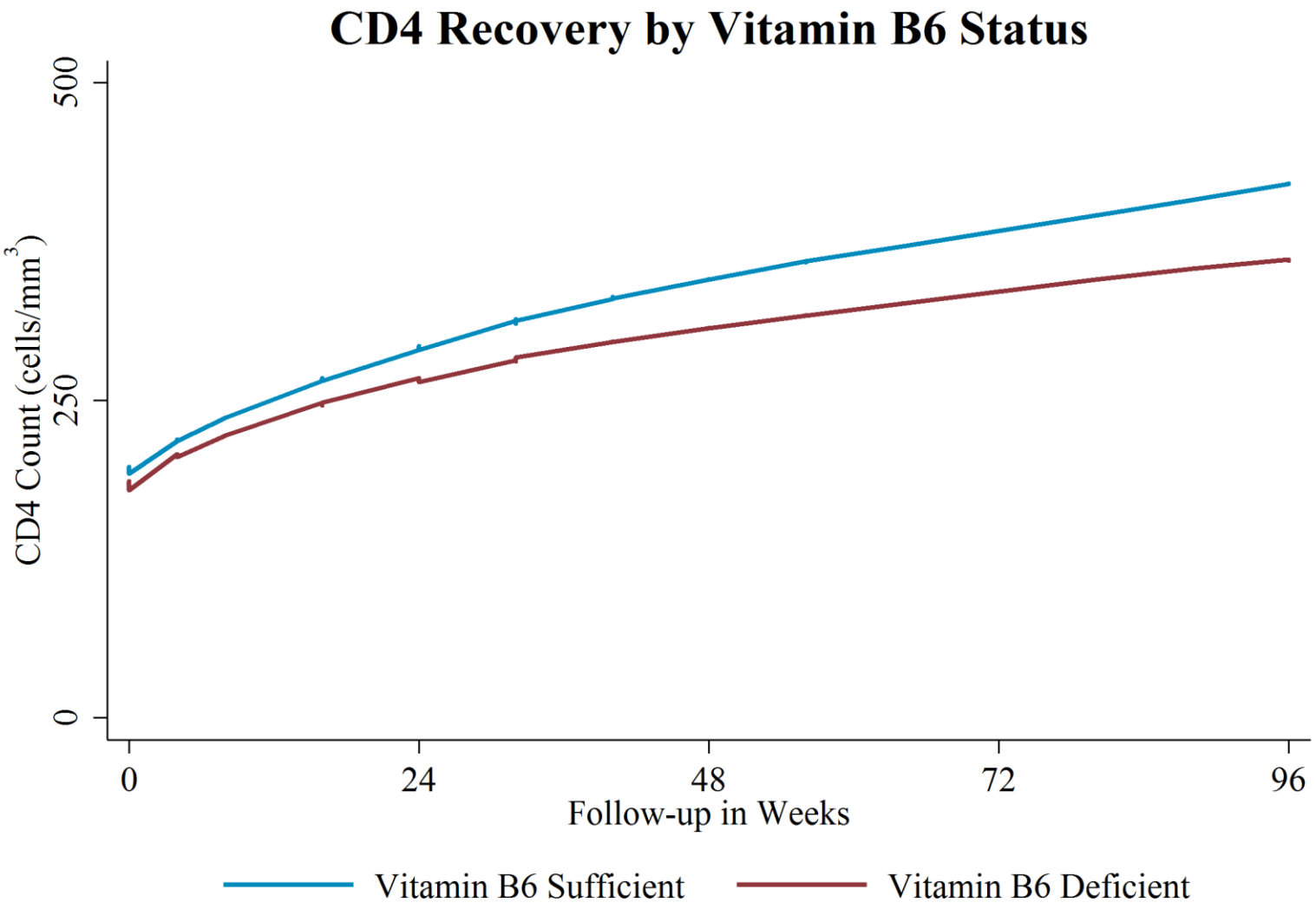

CD4 Recovery by Lycopene Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

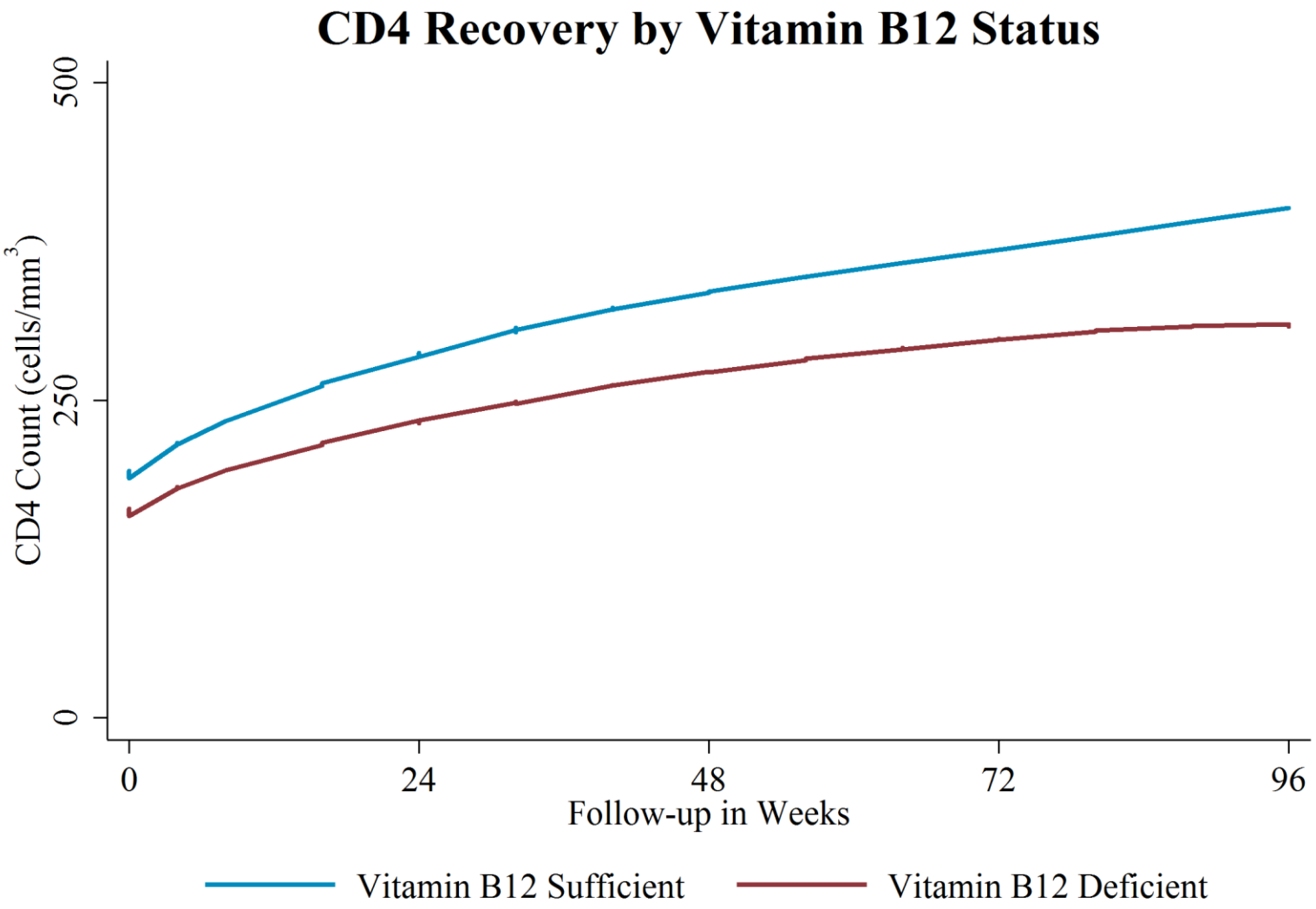

CD4 Recovery by Vitamin A (Retinol) Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Vitamin A Sufficient
Vitamin A Deficient

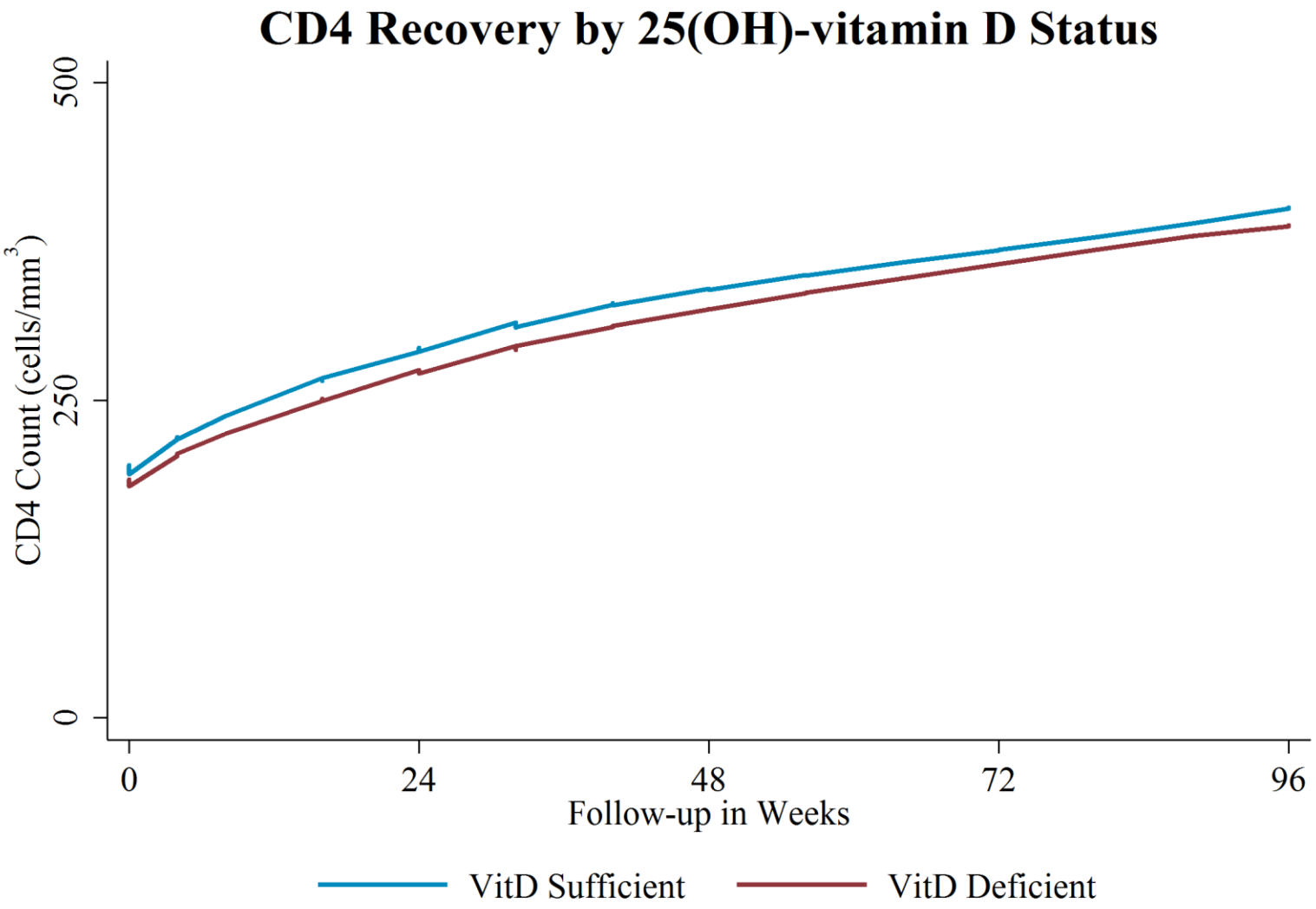

CD4 Recovery by Soluble CD14 Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)

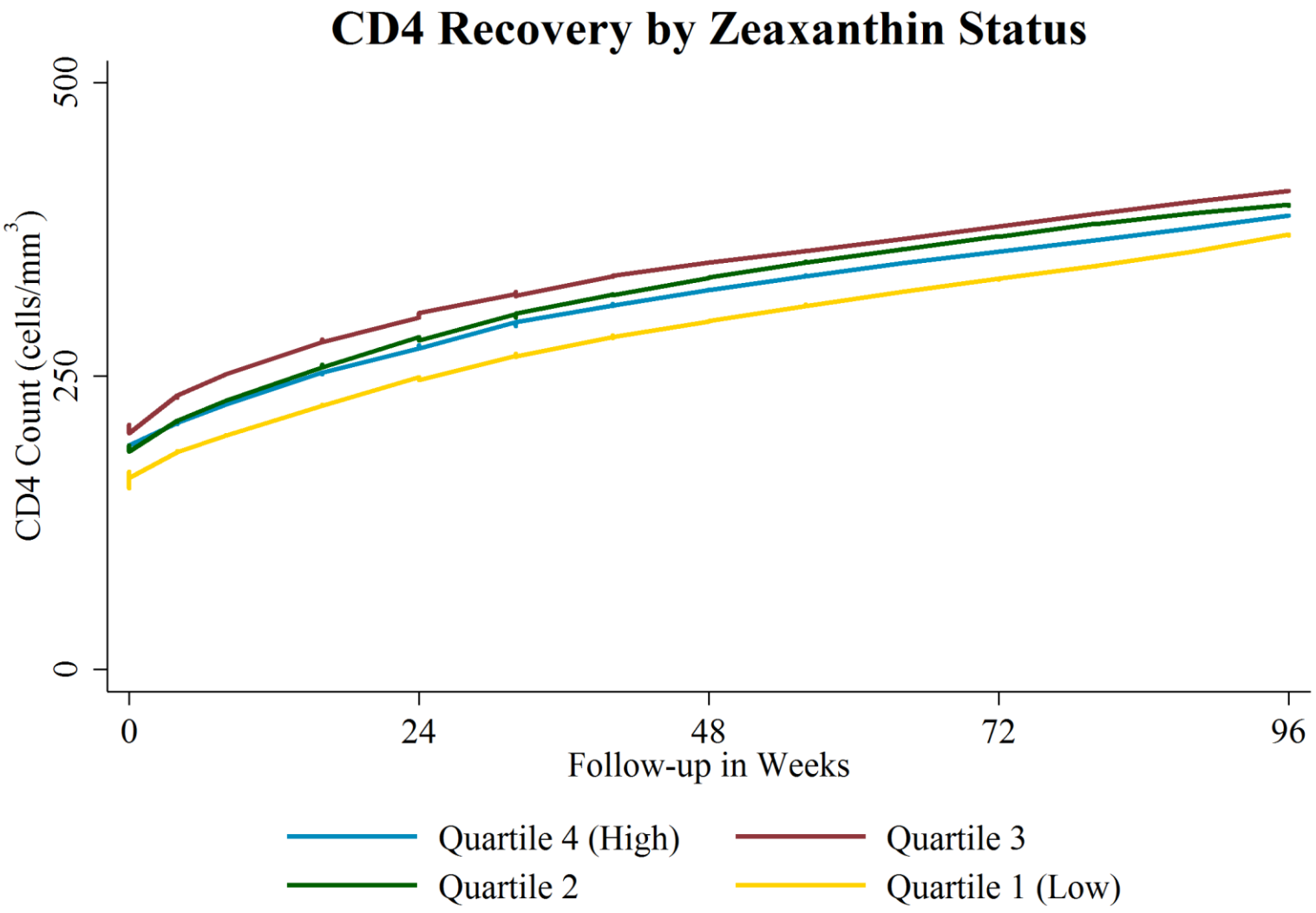

CD4+ Recovery by Selenium Status
CD4+ Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Selenium Sufficient
Selenium Deficient


CD4 Recovery by TNF-α Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 1 (Low)
Quartile 2
Quartile 3
Quartile 4 (High)


CD4 Recovery by Vitamin B6 Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Vitamin B6 Sufficient
Vitamin B6 Deficient


CD4 Recovery by Vitamin B12 Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Vitamin B12 Sufficient
Vitamin B12 Deficient


CD4 Recovery by 25(OH)-vitamin D Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
VitD Sufficient
VitD Deficient


CD4 Recovery by Zeaxanthin Status
CD4 Count (cells/mm3)
0
250
500
0
24
48
72
96
Follow-up in Weeks
Quartile 4 (High)
Quartile 3
Quartile 2
Quartile 1 (Low)

REFERENCES

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ERIN REBECCA EWALD

2935 St. Paul St. / Baltimore, MD 21218 / (704) 560-0310 / eewald@jhsph.edu

EDUCATION

ScM Infectious Disease Epidemiology, May 2014
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Thesis: *The impact of nutritional and inflammatory biomarker levels of CD4 recovery to 96 weeks in a treatment-naïve HIV-positive cohort*

Certificate in Field Epidemiology, December 2011
The University of North Carolina- Chapel Hill, Gillings School of Public Health, Chapel Hill, NC

A.B. Biological Sciences, June 2010
The University of Chicago, Chicago, IL
General Honors, Dean's List 2006-2007, 2009-2010

RESEARCH EXPERIENCE

Research Assistant, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
(June 2013—present)
- Analyze data from an AIDS clinical trial (n=1571) to investigate the relationship between pre-ART nutritional deficiencies and CD4 reconstitution over 96 weeks in collaboration with members of the Johns Hopkins Center for AIDS Research (CFAR)

Research Assistant, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
(October 2012—present)
- Process samples collected for Baltimore sites of the Multicenter AIDS Cohort Study (MACS) and the Women's Interagency HIV Study (WIHS), ongoing prospective studies of health histories of HIV-1 positive patients in multiple U.S. cities
- Record, enter, and store specimens in the Johns Hopkins Biological Repository via two databases

Research Technician, Johns Hopkins University, Baltimore, MD
(August 2010—August 2012)
- Research, develop, and execute experiments to determine the effects of environment on postnatal behavior, gene expression, and hormone levels in a rodent model
- Investigate the effects of maternal high-fat diet and stress on subsequent generations using a variety of molecular, genetic, and epigenetic techniques
- Present research at international conferences and departmental meetings

Research Assistant, The University of Chicago, Chicago, IL
(January 2010—September 2010)
- Read and analyze scientific papers on a variety of diseases including mental disorders, infectious diseases, and cancer to extract key statements
- Configure abstract statements to be put into a searchable database, focusing on genetics and epidemiology

HONORS AND AWARDS

Global Health Established Field Placement, Johns Hopkins Center for Global Health, Baltimore, MD (June 2013 – August 2013)

- Independent research award for tuberculosis risk factor research in an HIV-positive setting in Pune, India

TEACHING EXPERIENCE

Lead Teaching Assistant, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (September 2013 – present)

- Create syllabi, organize coursework and course schedule, and update lectures for four classes:
  Epidemiology and Public Health Impact of HIV and AIDS (~70 students)
  Advanced Topics on Control and Prevention of HIV/AIDS (~30 students)
  Epidemiology and Natural History of Human Viral Infections (~10 students)
  Stata Programming (~150 students)
- Grade homework, exams, and student presentations for the students enrolled in each class

Teaching Assistant, Public Health Studies, Johns Hopkins University, Baltimore, MD (September 2013 – present)

- Organize and independently lead discussion sessions with 25 students, hold weekly office hours, and respond to student questions about the undergraduate class *Fundamentals of Epidemiology*
- Grade exams, homework, online quizzes, and discussion exercises for the 85 students enrolled

Teaching Assistant, Biological Sciences Division, The University of Chicago, Chicago, IL (March 2010—June 2010)

- Organize and lead review sessions, create worksheets and homework assignments, and answer students' questions for an undergraduate biology class, *Eliminating Infectious Disease*
- Grade tests, worksheets, online forum discussions, and research papers for the 50 students enrolled

PUBLICATIONS

1. **Ewald ER**, Wand GS, Seifuddin F, Yang X, Tamashiro KL, Potash JB, Zandi P, Lee RS. (2014) Alterations in DNA Methylation of Fkbp5 as a Determinant of Blood-brain Correlation of Glucocorticoid Exposure. *Psychoneuroendocrinology* 44: 112-122.

2. Boersma GJ, Lee RS, Cordner ZA, **Ewald ER**, Purcell RH, Moghadam AA, Tamashiro KL. (2014). Prenatal stress decreases *Bdnf* expression and increases methylation of *Bdnf* exon IV in rats. *Epigenetics* 9(3): 437-447.

3. Sun B, Liang N, **Ewald ER**, Purcell RH, Boersma GJ, Yan J, Tamashiro KL. (2013) Early postweaning exercise improves central leptin sensitivity in offspring of rat dams fed high-fat diet during pregnancy and lactation. *AJP – Regulatory, Integrative, and Comparative Physiology* 305(9): R1076-84.

4. Yang X, **Ewald ER**, Huo Y, Tamashiro KL, Salvatori R, Sawa A, Wand GS, Lee RS. (2012) Glucocorticoid-induced loss of DNA methylation in non-neuronal cells and potential involvement of *DNMT1* in epigenetic regulation of *Fkbp5*. *BBRC* 420: 570-575.

SELECTED MEETING ABSTRACTS

1. **Ewald ER**, Seifuddin F, Yang X, Boersma GJ, Tamashiro KL, Wand GS, Lee RS (2012). Tissue-specific DNA methylation of *Fkbp5* as a determinant of blood-brain correlation of glucocorticoid exposure in mice. Neurobiology of Stress Workshop, Pittsburgh, PA.

2. Purcell RH, **Ewald ER**, Sun B, Volk K, Moran TH, and Tamashiro KL (2011). Mechanisms for metabolic side effects associated with the atypical antipsychotic olanzapine. Society for Neuroscience, Washington, D.C.

3. Boersma GJ, **Ewald ER**, Lee RS, Purcell RH, Sun B, Moran TH, and Tamashiro KL (2011). Prenatal environment influences expression and methylation of genes associated with emotionality and stress-coping. Anxiety & Depression: 21st Neuropharmacology Annual Conference, Alexandria, VA.

4. **Ewald ER**, Sun B, Purcell RH, Moran TH, and Tamashiro KL (2011). Maternal high fat diet alters liver gene expression in PND21 male rats. Society for the Study of Ingestive Behavior, Clearwater, FL.

VOLUNTEER EXPERIENCE

Disaster Action Team Leader, American Red Cross of the Chesapeake Region, Baltimore, MD (September 2012—present)

- Coordinate Red Cross volunteers to cover weekly shifts to respond to emergencies in Baltimore and surrounding areas

Disaster Action Team Member, American Red Cross of the Chesapeake Region, Baltimore, MD (January 2011—present)

- Work together with other team members to respond to local disasters, provide clients with immediate needs—food, clothing, shelter, etc.
- Participate in trainings in emergency preparedness, disaster public health, mental health, crisis response, and disaster assessment
- Respond to national disasters in the area (such as hurricanes and floods) as part of the Chesapeake region volunteer staff

Member, Alumni Schools Committee, The University of Chicago (August 2010—present)

- Interview prospective students in the Baltimore area, attend local prospective student events in order to aid the enrollment efforts of the admissions office

RELEVANT SKILLS

- Adept at statistical analysis software (**STATA, R, SAS**) and mapping programs (**ArcGIS**)
- Familiar with database management programs (**SQL, REDCap, Microsoft Access**)
- Adept at Microsoft Word, Excel, Powerpoint, and Publisher
- Proficient in Latin, intermediate-level Spanish.